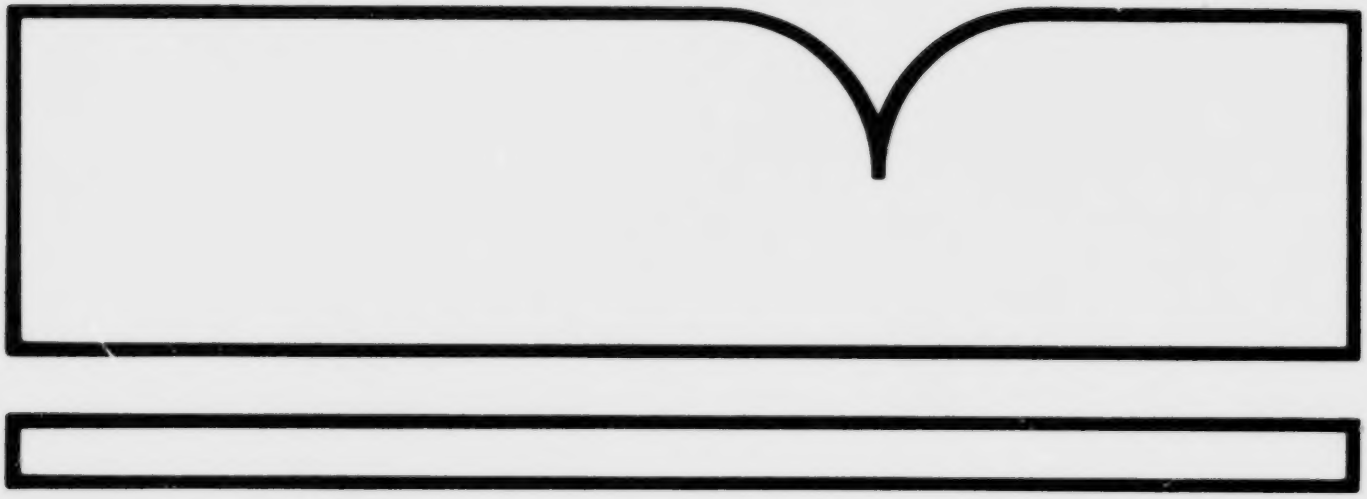
PB91-143412

Drinking Water Criteria Document on Carbofuran

Life Systems, Inc., Cleveland, OH

Prepared for:
Environmental Protection Agency, Washington, DC

28 Jun 90



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16. ABSTRACT

This document deals with the drinking water health effects of Carbofuran through the review of several studies. These studies include animal and humans. Physical and chemical properties are discussed. Carcinogenicity of the compound is reviewed and evaluated through various studies. Any existing guidelines and standards are presented.

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ICAIR
Life Systems, Inc.
Cleveland, OH 44122

for

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Office of Drinking Water
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Washington, DC 20460

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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish Maximum Contaminant Level Goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is non-enforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG for carbofuran. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity were evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document was comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature search in support of this document includes information published up to November 1983; however, more recent information may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (One-day, Ten-day and Longer-term, approximately 10% of an individual's lifetime) are included in this document. These values are not used in setting the MCL, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

Michael B. Cook
Director
Office of Drinking Water

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I. SUMMARY

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a carbamate pesticide introduced into agricultural use in the United States in 1968. It is used primarily for the control of insects on corn (soil application) and alfalfa (foliar application). Pure carbofuran is a white, crystalline solid with a water solubility of 700 ppm. In July 1987, a reassessment of carbofuran solubility by the manufacturer (FMC) resulted in a change of this value to 351 ppm at 25°C. It is formulated as 2%, 3%, 5%, 10% and 15% granules and as a 4 lb/gal aqueous suspension. Soil applications vary between 0.25 and 1 kg carbofuran/hectare, while foliar applications are 0.14 to 4.5 kg carbofuran/hectare.

Carbofuran is degraded in the environment both chemically and biologically by oxidative and hydrolytic mechanisms. Oxidative reactions lead to two important metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran. Both of these metabolites have toxicological properties comparable to those of the parent compound. Hydrolytic degradation of carbofuran results primarily in the removal of the methylcarbamyl moiety, giving carbofuran phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol). Structurally analogous phenolic metabolites are formed by hydrolysis of 3-hydroxycarbofuran and 3-ketocarbofuran. The toxicological result of such metabolism is loss of antiacetylcholinesterase properties, which essentially detoxifies the molecule.

The absorption, distribution and excretion of carbofuran in mammals are typical of those of other carbamate insecticides. Carbofuran is absorbed rapidly. Rats absorb 63.4% of an oral dose from the stomach in 30 minutes and 71.7% of a dermal dose within 15 minutes. Distribution to organs is rapid, with excretion of 70% of an oral dose in urine and 5% in feces in the first 24 hours.

Carbofuran is an acetylcholinesterase-inhibiting insecticide and the toxic symptoms in animals are typical of that mode of action. The acute oral LD_{50} values in mammals range from 2.0 to 14.1 mg/kg. Lethal effects following acute exposure are the result of acetylcholinesterase inhibition. Other acute effects on the immune system and the blood have been reported. These effects, however, are not lethal and appear to be readily reversible when exposure to the compound is terminated.

Acetylcholinesterase inhibition is the most sensitive indicator of the toxic effects of carbofuran. A 2.5-mg/kg dose administered orally to Sprague-Dawley rats resulted in definite symptoms of acute poisoning. An oral dose of 0.05 mg/kg resulted in only minimal effects on blood and liver acetylcholinesterase activity and no statistically significant effects on brain cholinesterase. An oral dose of 0.25 mg/kg resulted in minimal but statistically significant effects on brain cholinesterase. In chronic feeding studies, the No-Observed-Adverse-Effect Levels (NOAELs) were 1.0 mg/kg/day for rats and 3.0 mg/kg/day for mice, based on inhibition of acetylcholinesterase.

There have been many short-term assays of the mutagenic potential of carbofuran. There are a few positive results, particularly in the Ames test with Salmonella typhimurium strains TA98 and TA1538. Tests with other Salmonella strains and other short-term tests were negative. Carcinogenicity bioassays with rats and mice in two-year feeding studies resulted in no increase in tumor incidence in either species.

It is possible to prepare N-nitrosocarbofuran chemically by allowing carbofuran to react with nitrite in an acidic medium. Several in vitro studies have demonstrated the feasibility of this reaction, and tests of N-nitrosocarbofuran and the nitroso derivatives of 3-hydroxycarbofuran and 3-ketocarbofuran for mutagencity in Salmonella typhimurium strain TA100 gave positive results. These materials are among the most mutagenic compounds tested in the TA100 strain. N-Nitrosocarbofuran administered to rats resulted in increased numbers of stomach tumors. Of particular concern is the suggestion that the pH of the human stomach would favor the formation of N-nitrosocarbofuran, but there is only limited evidence that this reaction occurs in vivo.

The reproductive and teratogenic effects of carbofuran were assessed in beagle dogs, rats and rabbits. Some testicular degeneration was noted in beagles administered approximately 12.5 mg/kg/day carbofuran in the diet. In rats and rabbits, no-effect levels were 1.0 and 2.0 mg/kg/day, respectively. No terata were observed in any of these species.

In humans, dermal and oral exposure to carbofuran results in symptoms of cholinesterase depression, including nausea, stomach cramps, vomiting, increased salivation and tremors. In a controlled human experiment, single oral doses of carbofuran above 0.05 mg/kg/day resulted in symptoms of acetylcholinesterase inhibition.

The mechanism of action of carbofuran is that of a potent inhibitor of acetylcholinesterase. Carbofuran is a direct inhibitor, not requiring metabolic alteration to become active.

A NOAEL of 0.05 mg/kg/day, based on the absence of symptoms of acetylcholinesterase inhibition in humans, was used to calculate a One-day Health Advisory (HA) for the 10-kg child of 0.05 mg/L. The same value was used for the Ten-day HA since effects on acetylcholinesterase are reversible and do not appear to be cumulative. A NOAEL of 0.50 mg/kg/day, based on the absence of acetylcholinesterase inhibition and testicular degeneration in beagle dogs exposed via the diet for one year, was used to calculate a Longer-term HA for the 10-kg child of 0.05 mg/L and a Longer-term HA for the 70-kg adult of 0.18 mg/L. A Drinking Water Equivalent Level (DWEL) of 0.18 mg/L was calculated using the NOAEL of 0.50 mg/kg/day from the one-year feeding study in dogs.

The Office of Pesticide Programs has established tolerances for carbofuran in or on raw agricultural commodities. These tolerances are based on an Acceptable Daily Intake (ADI) of 0.005 mg/kg/day. The World Health

Organization has calculated an ADI of 0.01 mg/kg/day for carbofuran. For inhalation exposure in the work place, the time-weighted (8 hour/day) threshold limit value is 0.1 mg/m^3.

II. PHYSICAL AND CHEMICAL PROPERTIES

Synonyms for carbofuran include Furadan,[a] Curaterr,[a] Bay 70143, NIA 10242 and ENT 27164. Physical and chemical properties for carbofuran (chemical name, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) are listed in Table II-1. In addition to the reported water solubility of 700 mg/L at 25°C, Bowman and Sans (1979) determined a solubility of 320 mg/L at 19°C.

A. Manufacture and Use

Carbofuran is prepared by thermal rearrangement and cyclization of 2-methallyloxyphenol to 2,3-dihydro-2,2-dimethylbenzofuran-7-ol. This product is converted to the methyl carbamate by reaction with methyl isocyanate in the presence of triethylamine (Martin and Worthing 1977). Carbofuran is formulated as 2%, 3%, 5%, 10% and 15%[b] granules and as a 4 lb/gal aqueous suspension.[b] Furadan[a] is usually applied to foliage at 0.25 to 2 kg active ingredient/hectare, placed in or above seed furrows at 0.5 to 4 kg/hectare or broadcast at rates of 6 to 10 kg/hectare (Martin and Worthing 1977).

Carbofuran is a broad-spectrum carbamate insecticide, acaricide and nematocide. Carbofuran formulations have been used widely in efforts to

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[a] Registered trademark.

[b] FMC's communication.

Table II-1 Physical and Chemical Properties of Carbofuran

Property	Value
Chemical Abstracts Registry Service No.	1563-66-2
Synonyms	Furadan,[a] Curaterr,[a] Bay 70143, NIA 10242 ENT 27164
Chemical name	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate
Wiswesser Line Notation	T56 BOT&J C1 C1 IOVM1
Chemical formula	$C_{12}H_{15}NO_3$
Molecular weight	221.26
Elemental analysis	C, 65.14%; H, 6.83%; N, 6.33%; O, 21.69%
Physical state	White, crystalline solid
Odor	Odorless, slightly phenolic
Flammability	Not flammable; will support combustion if ignited
Explosive hazard	None
Corrosive action	None
Melting point	153° to 154°C
Density	1.180 (20°C)
Vapor pressure	6×10^{-7} mm Hg (25°C)[b]
Solubility % weight/weight, 25°C	
Acetone	15
Acetonitrile	14
Benzene	4
Cyclohexanone	9

continued-

[a] Registered trademark.
[b] Value for this parameter communicated to EPA by the registrant (FMC) based on their findings.

Table II-1 - continued

Property	Value
Dimethyl formamide	27
Dimethyl sulfoxide	25
Ethanol	4
Kerosene	<1
N-Methyl-2 pyrrolidone	30
Methyl chloride	12
Petroleum ether	<1
Xylene	<1
Water	0.035 (351 ppm)[a]

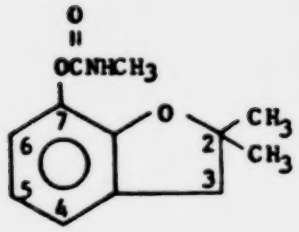
[a]Value for this parameter was communicated to EPA by the registrant (FMC) based on their findings.

Adapted from Windholz (1983), Kuhr and Dorough (1976), Midwest Research Institute (1976), and Cook (1973).

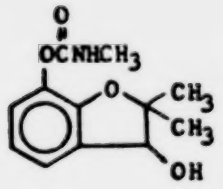
control corn rootworm, rice water weevils and mosquitoes, sugarcane nematodes and wireworms, as well as alfalfa weevil on alfalfa and thrips and nematodes on peanuts (Cook 1973). Although carbofuran is registered for use on many crops, its major use (91%) is in corn production, with 9% used on alfalfa and rice (based on 1974 data). It is registered as a contact insecticide for crops and as a systemic for soil treatment (Midwest Research Institute 1976). The first registered use for carbofuran in the United States was on field corn in 1968. The use of chlorinated hydrocarbons in the United States has decreased since the early 1970s, while other less persistent pesticides, including carbofuran, have been used more frequently. Domestic usage in 1972 was estimated by the Midwest Research Institute (1976) as 6.0 million pounds. By 1974 it was estimated that 6.8 million pounds was used on corn in the United States alone (Midwest Research Institute 1976). Although total use figures are not available, over 7 million pounds of carbofuran was used in 1974.

B. Environmental Fate and Stability

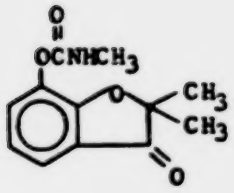
Carbofuran may undergo a number of chemical reactions in the environment, including photodecomposition, oxidation and hydrolysis (Midwest Research Institute 1976). Figure II-1 presents the structures and names of carbofuran and its main products. Cook (1973) stated that carbofuran hydrolyzes under alkaline or strongly acidic conditions, with cleavage at the carbamate linkage, but that hydrolysis does not occur in neutral or slightly acidic solutions. These statements are confirmed by the studies of Metcalf et al. (1968). The half-life of carbofuran (in phosphate buffer, pH 9.5 at 37°C) was



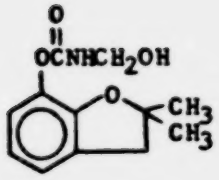
Carbofuran



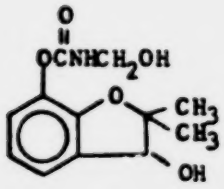
3-Hydroxycarbofuran



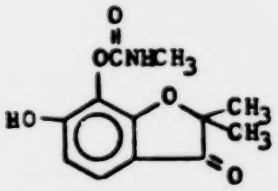
3-Ketocarbofuran



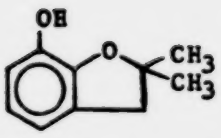
N-Hydroxymethyl carbofuran



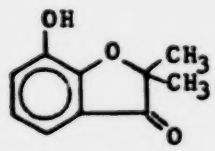
3-Hydroxy-N-hydroxymethyl carbofuran



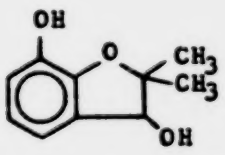
3-Keto-6-hydroxy-carbofuran (unconfirmed product)



Carbofuran phenol (2,2-dimethyl-7-hydroxy-2,3-dihydrobenzofuran) (2,3-dihydro-2,2-dimethyl-7-benzofuranol)



3-Ketocarbofuran phenol



3-Hydroxycarbofuran phenol

Adapted from Midwest Research Institute (1976).

Figure II-1 Chemical Structures of Carbofuran and Its Degradation Products

66.9 minutes. All carbofuran derivatives and metabolites were hydrolyzed faster than carbofuran under these conditions.

A matter of considerable environmental interest is the possibility of formation of N-nitroso derivatives of the N-methylcarbamate insecticides. The original work on this reaction utilized carbaryl, the most extensively used carbamate insecticide. Elespuru and Lijinsky (1973) demonstrated that carbaryl (0.5 or 0.1 M) plus nitrite (2.0 or 0.4 M) in aqueous solution containing 25% dimethyl sulfoxide reacted under acid conditions (pH 2.8 to 3.9) at 37°C to give yields of 5% to 11% nitrosocarbaryl after one hour. Similarly, Eisenbrand et al. (1975) produced nitrosocarbaryl by incubation of carbaryl with a five-fold molar excess of nitrite for 15 to 60 minutes in 0.1 N HCl (pH 1). Decreases in pH slightly increased the yield to a maximum of 3.2% in 1 N HCl.

Carbofuran undergoes a similar reaction to form N-nitrosocarbofuran (Seiler 1977, Lijinsky and Schmahl 1978, Nelson et al. 1980, 1981). The effect of pH on the formation (by nitrosation), stability (in vivo and in vitro) and degradative products of N-nitrosocarbofuran was observed by Rickard et al. (1982). The efficiency of nitrosation of carbofuran by nitrite in aqueous HCl solutions varied profoundly with moderate changes in pH. Reactions between carbofuran (0.5 µmol) and $NaNO_2$ (1.16 mmol, in one mL HCl solution) were allowed to proceed for ten minutes in the dark at 37°C. At pH 1 the yield of nitrosocarbofuran was 41.9%, at pH 2 the yield was down to 0.9%, and by pH 3 it had decreased to 0.01%. The authors suggested that these results indicated that the human stomach pH of 1 to 2 favors nitrosation much more heavily than does the rat stomach pH of 3 to 4.

Rickard et al. (1982) also investigated the stability of nitroso-carbofuran in buffered aqueous systems. At 37°C, nitrosocarbofuran was most stable between pH 3 (half-life of 470 minutes) and pH 5 (359 minutes) and much less so at pH 7 (70 minutes) or pH 1.5 (34 minutes). After two hours at pH 1.5, breakdown by denitrosation was heavily favored (62.9% carbofuran), but at pH 7 the major route was hydrolysis to the carbofuranphenol (31%). Two additional unidentified products (totaling 30.8%) were observed in the pH 7 sample. Breakdown at pH 3 or 5 led to similar quantities of carbofuran and carbofuran-phenol.

Kuhr and Dorough (1976) reported that carbofuran was applied at the equivalent rate of 1 pound/acre to freshwater model ecosystem aquaria, using both ring- and carbonyl-[^{14}C]-labeled compound. Following application to sorghum plants in this system, radiocarbon moved into the water, with peak concentrations appearing at seven days. Since the levels from the ring-labeled carbofuran were much higher than those from the carbonyl-labeled preparation, it was concluded that hydrolysis of the methylcarbamate moiety had occurred with release of $^{14}CO_2$. There are no reports in the literature of levels of carbofuran in water under natural conditions, nor has it occurred in drinking water.

C. Summary

Carbofuran is a broad spectrum carbamate insecticide, acaracide and nematocide, which has enjoyed wide agricultural use in the United States,

particularly on corn. Its solubility in water is low, reported by different authors to be 320 and 700 mg/L; FMC's most recent value is 351 ppm at 25°C. It is moderately soluble in organic solvents. Pure carbofuran is a white crystalline solid with a slightly phenolic odor. It is synthesized by the thermal rearrangement and cyclization of 2-methallyloxyphenol to 2,3-dihydro-2,2-dimethyl-7-nitrobenzofuran, reduction to the amine, diazotization, and conversion to the phenol; followed by reaction with methyl isocyanate in the presence of triethylamine to form the methylcarbamate. Under alkaline conditions the compound is readily hydrolyzed in water. Carbofuran has not been detected in drinking water in the United States. It has been shown that carbofuran is nitrosated by nitrite in acidic media to form nitrosocarbofuran.

III. TOXICOKINETICS

A. Absorption

Ahdaya et al. (1981) studied the absorption and distribution of radioactive ^{14}C-labeled carbofuran (1 µCi per 1 mg/kg dose), which was administered at 1 mg/kg in 0.1 mL carrier (Emulphor:ethanol:water, 1:1:8) via stomach feeding needle to fasted female ICR mice (seven to eight weeks old, 25 to 30 g). The mice were sacrificed at intervals of 1, 5, 15, 30 and 60 minutes after administration (three mice per interval), and the amount of unabsorbed radioactivity in the gastrointestinal tract was determined. Also, absorbed radioactivity was determined for selected organs by scintillation counting. The results, shown in Table III-1, indicated that 51.2% of the radioactivity was absorbed by 15 minutes and 67.4% by 60 minutes.

Ahdaya and Guthrie (1982) studied the stomach absorption of carbofuran using the same procedures as Ahdaya et al. (1981), but the stomachs of the mice were surgically ligated at the pylorus. Approximately 19% of the radioactive carbofuran was absorbed within one hour. Thus, the stomach alone is a significant route of absorption, at least when ligated. Compared to the gastrointestinal absorption obtained by Ahdaya et al. (1981), stomach absorption was about 28% of total absorption. The amounts of carbofuran recovered from the intestine (2.6%) must have been through intestinal secretion, since the pylorus was ligated.

Table III-1 Absorption and Recovery of Radioactive-Labeled Oral Doses of Carbofuran Intubated in Mouse Stomachs

Time	% Absorption[a]	% Administered radioactivity recovered	
		CO_2	Urine
1 min	34.5	-	-
5 min	44.7	-	-
15 min	51.2	1.1	5.6
30 min	63.4	3.0	17.1
60 min	67.4	6.0	24.0

[a]Absorption was calculated as the quantity: (100% - % in gastrointestinal tract).

Adapted from Ahdaya et al. (1981).

Shah et al. (1981) studied the dermal absorption of carbofuran in seven- to eight-week-old female ICR mice. Doses of ^{14}C-labeled (1 uCi per 1 mg/kg dose) carbofuran were applied in 0.1 mL acetone to a 1-cm^2 shaved area of the upper shoulder skin of the mice. Mice were sacrificed at 1, 5, 15, 60 and 480 minutes after application. Absorption, as measured by loss of radioactivity from the skin at the site of application, was determined by scintillation counting. The results (Table III-2) show that about 33% was absorbed by five minutes, 76% by 60 minutes and 95% by 480 minutes after administration.

B. Distribution

Ahdaya and Guthrie (1982) ligated mouse stomachs at the pylorus prior to administration of ^{14}C-labeled carbofuran and monitored distribution to various organs and tissues. The distribution to organs was related to stomach absorption, in this study, since the stomach was ligated. The results are shown in Table III-3.

Shah et al. (1981) examined the time course of distribution of ^{14}C-labeled carbofuran to organs and tissues following dermal exposure. Results are shown in Table III-4. Immediately apparent in comparing Tables III-3 and III-4 is the very rapid entry of carbofuran into the organism and its subsequent distribution in blood to other organs. Table III-5 shows the distribution in organs and tissues after 480 minutes (dermal exposure). FMC believes that the vast majority of the absorbed material is also excreted rapidly, as noted by the material balance in Table III-5. However, as noted in Table III-4, the total percentage of ^{14}C-labeled carbofuran remaining in the carcass after

Table III-2 Dermal Penetration After Topical Application of ^{14}C-Carbofuran in Mice

Time (min)	% Penetration[a]
1	28.5
5	32.6
15	71.7
60	76.1
480	94.7

[a]Values expressed as geometric mean of three measurements.

Adapted from Shah et al. (1981).

Table III-3 ^{14}C-Carbofuran Recovered in Organs and Tissues Following Absorption Through the Ligated Stomach of Mice

Time	Liver	Blood	Stomach	Intestine	Urine	Carcass
15 min	0.4[a]	0.4	2.8	1.0	0.6	3.8
30 min	0.7	0.6	4.1	2.2	2.7	4.7
60 min	0.9	1.2	4.0	2.6	5.3	4.9

[a]Values are % of total absorbed dose.

Adapted from Ahdaya and Guthrie (1982).

Table III-4 Distribution of ^{14}C-Labeled Carbofuran in Organs and Tissues Following Dermal Absorption in Mice

Organ/ Tissue	Time after administration		
	5 min	15 min	60 min
Liver	0.1[a]	2.4	0.6
Blood	<0.1	1.7	0.5
Fat	<0.1	0.2	<0.1
Excretory Products[b]	0.1	0.4	8.3
Carcass[c]	32.4	67.3	66.4

[a]Values expressed as % ^{14}C recovered, geometric mean of samples.
[b]Includes urine, CO_2 and feces.
[c]Carcass = portion of body remaining after removal of tissues and organs, as detailed in Table III-5.

Adapted from Shah et al. (1981).

Table III-5 Geometric Mean of Percent Recovered ^{14}C, 480 Minutes After Topical Application of ^{14}C-Carbofuran to Mouse Skin[a]

Organ/Tissue	%
Heart	0.1
Lungs	0.1
Brain	0.1
Kidney	0.3
Bladder	<0.1
Fat	0.2
Ear	0.3
Spleen	<0.1
Bone marrow	0.3
Muscles	0.6
Stomach	3.3
Intestine	2.6
Liver	1.0
Blood	0.8
Excretory products	72.8[b]
Carcass[c]	11.9

[a] A dose of 1 mg ^{14}C-carbofuran/kg was applied in acetone solution to shaved skin (upper shoulder) of ICR mice.

[b] Approximately 1/3 urine, 2/3 feces.

[c] Carcass = portion of body remaining after removal of tissues and organs listed.

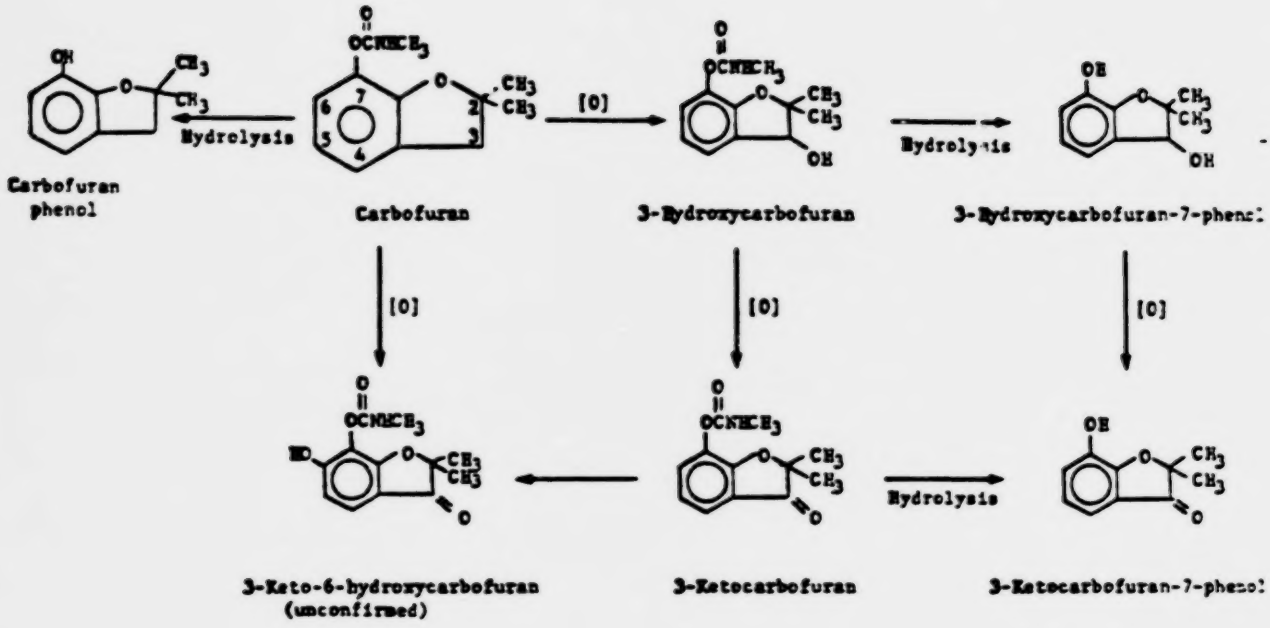
Adapted from Shah et al. (1981).

60 minutes of administration is 66.4%. This result also indicates that carbofuran is easily absorbed after dermal exposure.

C. Metabolism

The main pathway of metabolism of carbofuran in plants, insects, rats and mice appears to consist of hydroxylation at the benzylic carbon to give 3-hydroxycarbofuran (see Figure III-1). Further oxidation yields 3-ketocarbofuran, followed by hydrolysis and conjugation. Carbofuran can be hydrolyzed to carbofuran phenol, or hydrolysis can follow oxidation to yield 3-hydroxycarbofuran phenol, and 3-ketocarbofuran-7-phenol (Dorough 1968, Metcalf et al. 1968, Knaak 1971, Midwest Research Institute 1976). Hydrolysis is a significant pathway in mammals, but is minor in insects and plants.

Dorough (1968) studied the metabolites of carbofuran formed after incubating the ^{14}C-ring-labeled compound with rat liver homogenate 15,000 x g supernatant fraction. The results are shown in Table III-6. The main metabolites identified were, in order of recovery, 3-hydroxycarbofuran, N-hydroxymethylcarbofuran, carbofuran phenol, 3-hydroxy-N-hydroxymethylcarbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol and 3-ketocarbofuran phenol. The rat liver homogenate 15,000 x g supernatant fraction was the most efficient liver enzyme fraction in metabolizing carbofuran. This fraction metabolized 36.2% of the added compound based on calculations with ^{14}C-carbonyl-carbofuran. The 105,000 x g pellet (microsomes) metabolized 11.8%, and the 105,000 x g supernatant metabolized 5.9%. In all three cases, nicotinamide adenine dinucleotide phosphate ($NADPH_2$) was necessary for activity, indicating the involvement of mixed function oxidases in carbofuran metabolism.



Adapted from Midwest Research Institute (1976) and Metcalf et al. (1968).

Figure III-1 Oxidation and Hydrolysis of Carbofuran

Table III-6 Metabolism of ^{14}C-Ring-Labeled Carbofuran by Rat Liver Homogenate 15,000 x g Supernatant Fraction

Metabolite	Yield (% Radioactivity added)
3-Hydroxy-N-hydroxymethylcarbofuran	2.07
N-Hydroxymethylcarbofuran	7.05
3-Hydroxycarbofuran	20.13
3-Ketocarbofuran	0.22
Carbofuran	58.50
3-Hydroxycarbofuran phenol	0.02
3-Ketocarbofuran phenol	0.02
Carbofuran phenol	3.82
Water soluble radioactivity	7.01
Unknown I	0.10
Unknown II	0.09
Unknown III	0.03
Total Recovery	99.06

Adapted from Dorough (1968).

The 3-hydroxy-N-hydroxymethylcarbofuran, when incubated with the rat liver homogenate 15,000 x g supernatant fraction, was converted mainly to water-soluble conjugates, presumably by initial hydrolysis of the carbamyl group. Carbofuran phenol also was metabolized by the rat liver enzymes to conjugated water-soluble compounds. N-Hydroxymethyl-carbofuran was metabolized to 23.9% water-soluble materials, 6.9% 3-hydroxy-N-hydroxymethylcarbofuran, 4.1% 3-hydroxycarbofuran phenol and 2.2% unidentified compounds. The 3-hydroxycarbofuran was metabolized to 23.4% water-soluble materials, 3.9% 3-hydroxycarbofuran phenol, 8.9% 3-hydroxy-N-hydroxymethylcarbofuran and 0.6% unidentified compounds. These same metabolites also were detected in the urine of rats treated orally with ^{14}C-carbofuran, mostly in the form of conjugates (Dorough 1968). The quantities of recovered metabolites are shown in Table III-7.

Dorough (1983) studied the metabolism in rats of 3-hydroxycarbofuran glucoside, a plant metabolite of carbofuran. This compound is a major residue in many plants treated with carbofuran. The residual life of 3-hydroxycarbofuran in rats was longer when administered as the glucoside conjugate than as the free metabolite. It was, however, demonstrated that the further metabolism of the glucoside and the free metabolite yielded the same metabolites in bile, although metabolism of the glucoside was slower. The glucoside was cleaved in the gastrointestinal tract to 3-hydroxycarbofuran, which the animal then metabolized further, including producing 3-hydroxycarbofuran glucuronide. Although 3-hydroxycarbofuran is of similar acute toxicity to rats as carbofuran, the toxicological significance of exposure of the

Table III-7 Metabolites Recovered in Urine of Rats Following Acid Hydrolysis of Water-Soluble ^{14}C Products Recovered from Oral Treatment With Ring ^{14}C-Labeled Carbofuran

Metabolite	% of Total ^{14}C-Water Solubles in Urine[a]
3-Hydroxy-N-hydroxymethylcarbofuran	4.02
3-Hydroxycarbofuran	14.78
3-Hydroxycarbofuran phenol	1.43
3-Ketocarbofuran phenol	50.54
Carbofuran phenol	21.12
Unknown	0.04
Water soluble radioactivity[b]	8.07
Total recovery	100.00

[a]Urine from rats collected during the 72-hour period following oral dosing of 4 mg/kg carbofuran. Greater than 90% of the radioactivity in the urine could not be extracted with chloroform.

[b]^{14}C-Materials remaining in the aqueous layer after acid treatment and extraction with chloroform.

Adapted from Dorough (1968).

animal to the glucoside is unknown. Glucoside becomes bioavailable at a slower rate than aglycone.

The anticholinesterase properties of the three metabolites with intact carbamyl-moieties were estimated colorimetrically using housefly heads as the enzyme source (Dorough 1968). All were less potent than carbofuran itself, (molar I_{50} value, 3.3×10^{-8}). Some anticholinesterase activity was apparent for 3-hydroxycarbofuran (2.1×10^{-1}), 3-ketocarbofuran (3.3×10^{-7}) and 3-hydroxy-N-hydroxy-methylcarbofuran (1.4×10^{-6}).

Rickard and Dorough (1984) investigated nitrosocarbofuran formation in the stomach of rats and guinea pigs in vivo. Female Sprague-Dawley rats (200 to 250 g) or Hartley guinea pigs (200 to 250 g) were given 0.50 µmol ^{14}C-labeled carbofuran in 0.1 mL ethanol by stomach tube, followed immediately by 1.16 mmol sodium nitrite in 0.3 mL water. After 15 minutes, the contents of the stomach were removed, extracted with ether, fractionated by two-dimensional thin-layer chromatography and ^{14}C-nitrosocarbofuran was quantitated by direct liquid scintillation of the gel. Only low levels of nitrosocarbofuran were detected: 0.65% in guinea pig stomach and 0.03% in rat stomach. The authors indicated that recovery of nitrosocarbofuran was only about 50% during the extraction procedure, but that the low yields were probably related to poor mixing of reactants in the stomach as well as absorption of reactants and/or the products. Similar results were obtained when carbofuran and sodium nitrite were given in feed. Mixing carbofuran and sodium nitrite with gastric contents in vitro resulted in higher yeilds: 30% nitrosation with guinea pig and 0.5% with rat stomach contents. The

difference was attributed to the pH of the gastric contents (pH 1 to 2 for the guinea pig and pH 3 to 5 for the rat). Since the pH in human stomach is also about 1 to 2, the authors concluded that nitrosation of carbofuran in humans would be similar to that in guinea pigs.

N-Nitrosocarbofuran was administered to rats by stomach tube, followed by immediate sacrifice and recovery of labeled material (Rickard et al. 1982). The major form recovered was nitrosocarbofuran (42.9%). The authors observed substantial hydrolysis (14.5% carbofuran phenol), while denitrosation (0.8% carbofuran) was only a minor route of breakdown. Some material (3.6%) was water soluble, and 24.1% was unextracted by ether, giving a total recovery of 89.3% of the administered dose.

D. Excretion

Dorough (1968) investigated the elimination of ring ^{14}C-labeled carbofuran after oral administration of a 4.0-mg/kg dose to 200-g rats (sex and strain not specified). The amounts found in urine and feces at succeeding time intervals after treatment are shown in Table III-8. Excretion of carbofuran was rapid, with 74.5% being eliminated in the first 24 hours after administration. Hurst and Dorough (1978) showed that the half-time for elimination of carbofuran increased linearly with increased dosage in rats.

In their study of ^{14}C-labeled carbofuran administered to fasted mice via stomach feeding needle, Ahdaya et al. (1981) reported that after 60 minutes,

Table III-8 Elimination of Radioactivity From Rats Treated Orally With Ring ^{14}C-Labeled Carbofuran

Hours after treatment	Cumulative % of administered dose	
	Urine	Feces
2	5.9	0.3
4	15.0	0.5
6	21.4	0.5
12	37.8	0.9
24	72.2	2.3
32	87.7	2.4
48	89.0	2.5
56	89.7	3.0
72	90.8	3.3[a]
96	91.4	--
120	91.6[a]	--
	Total:	94.9%

[a]Last sample containing radioactivity.

Adapted from Dorough (1968).

6% and 24% of the radioactively labeled doses were observed in CO_2 and urine, respectively (see Table III-1).

Shah et al. (1981) examined excretion in their study of the distribution of ^{14}C-ring-labeled carbofuran in young female mice. Eight hours after dermal administration, 72.8% was excreted; approximately two-thirds in feces and one-third in urine. Thus, 48.7% of the total dose applied to the animals was excreted in feces (67% x 72.8%).

Marshall and Dorough (1984) investigated the excretion of 3-hydroxycarbofuran, one of the major metabolites of carbofuran. Ring-labeled ^{14}C-3-hydroxycarbofuran was administered by intubation to male Sprague-Dawley rats. After 1.5 hours, 40% of the label was exreted in urine and at 6 hours, 79% had been excreted in urine. Both glucuronide and sulfate conjugates were major components of the urinary radiolabeled material, indicating that biliary metabolism was important in the excretory pathway.

E. Bioaccumulation and Retention

No data regarding tissue levels as a function of time in a long-term exposure study were found in the literature. However, the data summarized in Section C (Metabolism) indicate that carbofuran is metabolized rapidly in the body, and the data summarized in Section D (Excretion) indicate that carbofuran is excreted efficiently from the body in feces, urine and expired air. Yu et al. (1974) showed that carbofuran does not accumulate in terrestrial or aquatic organisms in a model ecosystem.

F. Summary

The absorption, distribution and excretion of carbofuran are typical of those of other carbamate insecticides. Carbofuran is rapidly absorbed. In rats, 67.4% of an oral dose and 76% of a dermal dose were absorbed in 60 minutes. Distribution to various tissues and organs is also rapid. Carbofuran is metabolized by oxidative and hydrolytic pathways to yield metabolites which undergo conjugation to form water-soluble products that are rapidly excreted. Rats excrete about 75% of an orally administered dose within the first 24 hours, primarily in urine (70%), with lesser amounts (5%) in feces. In mice, about one-third of a dermal dose is excreted in urine, and two-thirds in feces.

IV. HUMAN EXPOSURE

To be provided by the Science and Technology Branch, ODW, EPA.

V. HEALTH EFFECTS IN ANIMALS

A. Acute Toxicity

The acute toxic effects, especially lethality, resulting from exposure to carbofuran are largely attributable to inhibition of the enzyme acetylcholinesterase, the ultimate cause of death being respiratory failure. This enzyme is an essential component in the synaptic transmission of nerve impulses in all animals, including humans and insects. Unlike some acetylcholinesterase inhibitors, carbofuran acts directly and does not require metabolic alteration. Some organophosphates have a latent period of several hours between absorption and appearance of signs of toxicity, whereas carbofuran, being a direct inhibitor, produces signs and symptoms within 30 minutes after absorption (Tobin 1970).

1. Lethality

The data from the published and unpublished studies of the lethal effects of single oral and dermal doses of carbofuran are listed in Table V-1. Numerous unpublished studies on the acute toxicity of carbofuran conducted by the FMC Corporation are cited in the review by the Midwest Research Institute (1976). The oral LD_{50} ranged from 6.4 to 14.1 mg/kg in rats, and from 25.0 to 38.9 mg/kg for the white Leghorn chicken.

Table V-1 Acute Oral Toxicity of Carbofuran

Species	Effect	Dose (mg/kg)	Reference
Rat	8/32[a]	2.5	Cambon et al. 1979
Rat[b]	LD_{50}	6.4 - 14.1	Midwest Research Institute 1976
Mouse	LD_{50}	2.0	Fahmy et al. 1970
Dog[b]	2/10[a]	18.85	Midwest Research Institute 1976
Chick (10 to 12 days old)	LD_{50}	6.3	Sherman et al. 1967
Chicken[b]	LD_{50}	25.0 - 38.9	Midwest Research Institute 1976
Mallard (adult)	LD_{50}	0.415 0.7 (male) 0.86 (female)	Hudson et al. 1972 FMC Corp. 1972
Fulvous Whistling Ducks	LD_{50}	0.238	Tucker and Crabtree 1970
Bobwhite quail	LD_{50}	8.0	FMC Corp. 1972
Japanese quail	LD_{50}	4.9 (male) 3.5 (female)	FMC Corp. 1972
Pheasant	LD_{50}	4.0 (male) 6.2 (female)	FMC Corp. 1972
Quelea	LD_{50}	0.42	Schafer et al. 1973
House Sparrow	LD_{50}	1.3	Schafer et al. 1973
Red-winged Blackbird	LD_{50}	0.42	Schafer et al. 1973

[a]Number dead/number exposed.
[b]Numbers reported without documentation from unpublished studies.

The beagle dog appeared more resistant than the rat. Chickens were more resistant than other bird species. The data on wildlife are presented because of the paucity of published, peer-reviewed studies on laboratory animals.

Wolfe and Esher (1980) determined lethal levels of carbofuran in mice at dietary levels of 250 and 500 mg/kg, or approximate daily doses of 49 or 98 mg/kg bw, calculated using a reported body weight of 13.8 g and a daily food consumption of 2.7 g/day. After four days, mortality was 20% at the 250-mg/kg (49 mg/kg/day) dose level and 100% at the 500-mg/kg (98 mg/kg/day) level.

Tobin (1970) reported a dermal LD_{50} value in rabbits of 10,200 mg/kg for technical grade carbofuran and >10,000 mg/kg for 10% granular carbofuran. The acute dermal LD_{50} in rabbits was much lower (14.7 mg/kg) when carbofuran was applied in organic solvent (Dowanol DMP) (Midwest Research Institute 1976).

A summary of acute inhalation lethality data is given in Table V-2.

2. General Effects

The toxic effects of carbofuran are a result of its inhibition of the enzyme acetylcholinesterase. Mount and Oehme (1981) studied the effect of carbofuran and other pesticides on brain cholinesterase activity in cattle, swine and sheep. Brains of cattle (one year to adult) were removed at slaughter and homogenized for cholinesterase assay. A number of the animals

Table V-2 Inhalation Acute Toxicity Studies of Carbofuran[a]

Test animal	Sex	Formulation	Dust or aerosol	LD_{50}[b]
Rat	Mixed	80 WP	Dust	85
	Mixed	50 WP	Dust	120
	Male	50 WP	Dust	108
	Female	50 WP	Dust	133
Guinea Pig	Mixed	50 WP	Dust	74
	Male	50 WP	Dust	74
	Female	50 WP	Dust	53
	Mixed	75 WP	Aerosol	53
	Male	75 WP	Aerosol	43
	Female	75 WP	Aerosol	65
	Mixed	75 WP	Dust	43
	Male	75 WP	Dust	53
	Female	75 WP	Dust	45
Dog	Male	50 WP	Dust	52

[a]Experimental details are not provided for these otherwise unpublished studies.
[b]In mg/m^3 air.

Adapted from Tobin (1970).

were known to be "poisoned" with carbofuran (dose unspecified). Brain cholinesterase activity from these poisoned animals was depressed 71% compared to control animals.

Despite the fact that the mechanism of action of carbofuran is acetylcholinesterase inhibition within the nervous system and at the neuromuscular junction, most studies use cholinesterase activity in the blood as an indicator. Cambon et al. (1979) administered carbofuran to groups of pregnant Sprague-Dawley rats on gestation day 18. Eight animals per group were administered either 0.05, 0.25 or 2.5 mg/kg carbofuran by gastric intubation. The rats were killed 1, 5 or 24 hours after dosing and the activity of acetylcholinesterase was measured in both fetal and maternal blood, brain and liver. Toxic signs (tremors, salivation, miosis, dypsnea, piloerection) appeared at the highest dose (2.5 mg/kg) within five minutes. Mortality was high in this group. Table V-3 summarizes the results after one hour. The activity of acetylcholinesterase was significantly ($P<0.05$) reduced in all maternal and fetal tissue samples taken from surviving dams one hour after treatment with 2.5 mg/kg carbofuran. For lower doses, inhibition was found only in certain tissues one hour after treatment. There was a semiquantitative correlation between acetylcholinesterase inhibition in blood and in brain (nervous system). Cambon et al. (1980) observed differential inhibition of isoenzymes of acetylcholinesterase in both maternal and fetal brain. This study defines a LOAEL of 0.05 mg/kg for a single dose based on inhibition of maternal and fetal blood acetylcholinesterase and maternal liver acetylcholinesterase.

Table V-3 Activity of Acetylcholinesterase in Maternal and Fetal Tissues From Rats Administered Carbofuran by Gastric Intubation

Dose (mg/kg)	Time	Acetylcholinesterase units per gram protein		Acetylcholinesterase units per gram of organ			
		Maternal blood	Fetal blood	Maternal brain	Maternal liver	Fetal brain	Fetal liver
Control	18 days	22.9±1.47	24.0±2.42	9.41±1.45	5.89±0.86	1.45±0.06	1.75±0.07
	19 days	23.7±1.06	17.7±1.48	9.30±1.70	3.70±1.18	1.39±0.10	1.35±0.11
0.05	1 hour	19.6±2.33[a]	16.4±3.70[b]	7.93±1.68	4.08±0.87[b]	1.42±0.11	1.81±0.22
0.25	1 hour	19.3±2.62[a]	17.5±3.15[b]	6.46±1.28[a]	5.43±1.06	1.45±0.06	1.44±0.22[a]
	5 hours	22.2±2.17	19.7±4.78[c]	8.16±2.00	4.60±0.95[c]	1.57±0.17	1.37±0.20[b]
	24 hours	23.6±3.49	18.7±3.97	7.42±1.25	4.84±0.40	1.55±0.02	1.38±0.09
2.50	30 min		14.7±2.84[b]	2.54±0.48[b]		1.17±0.11[b]	1.07±0.13[b]
	1 hour	18.3±2.18[b]	14.9±1.38[b]	4.11±1.03[b]	4.66±0.97[c]	1.24±0.15[c]	1.08±0.36[b]
	5 hours	21.0±1.53[c]	19.2±5.11[c]	6.49±2.40[c]	4.06±0.75[b]	1.27±0.30	1.37±0.25[a]
	24 hours	24.3±2.85	15.8±2.39	8.44±2.03	4.56±1.13	1.77±0.36	1.47±0.16

[a]Significantly different from control at P<0.01.
[b]Significantly different from control at P<0.001.
[c]Significantly different from control at P<0.05.

Adapted from Cambon et al. (1979).

A number of researchers have explored the potential of carbofuran to affect various biochemical parameters other than acetylcholinesterase. Cranmer et al. (1978) measured several endocrine system parameters in mice exposed *in utero* to two levels of carbofuran. Adult female F_2 hybrid mice were fed daily doses of 0.01 or 0.50 mg/kg bw carbofuran (technical grade) in peanut butter. Doses chosen were 0.02% and 1% of the minimum dose, which caused increased terata or morbidity in pups as determined in a pilot study. Treatment began the day after mating and continued throughout the entire gestation period (22 days). Dams of all groups gave birth to approximately equal numbers of normal offspring. Exposure of pups through mother's milk may have occurred, since this was not controlled. At 101 days of age, pups exposed *in utero* were sacrificed for endocrinological evaluation. Levels of plasma corticosterone were measured in resting mice and mice stressed by swimming in 2°C water. *In vitro* corticosterone production by excised adrenals was measured for resting mice. Liver capacity to metabolize exogenous corticosterone was measured three ways: as side-chain reduction (by the blue tetrazolium (BT) reaction), as A-ring reduction (by UV absorption) or as the two together (by the acid fluorescence technique). Two independent control groups were examined concurrently. Group size was 8 to 11 mice except for the stressed groups, which had 3 to 5 mice. Differences in group means were detected by either Student's t-test or Duncan's multiple range test. Significant differences had P values below 0.05.

No differences were observed in any of the high-dose mice, male or female. The low-dose females showed increased resting plasma corticosterone levels and increased liver weight (which was proportional to the observed body

weight increase). Low-dose females also exhibited decreased total liver cortisone metabolic activity, but neither individual metabolic route differed significantly from the controls. The authors stated that results obtained by the fluorescence technique were not necessarily equal to the sum of the UV or BT values. The low-dose males exhibited decreased liver metabolic activity by all three measures. While the reasons for lack of a dose-response relationship in this experiment are unclear, the authors maintained that such complex dose-response curves reflect a real biological phenomenon. There are a number of examples in the literature of such dose-response inversions following prenatal exposure to toxic compounds (Cranmer et al. 1978).

Several studies have investigated the interaction of carbofuran with the immune system. Street and Sharma (1975) administered carbofuran in feed to groups of seven male New Zealand rabbits (2 kg). Doses of 0, 0.03, 0.16, 0.49 or 1.05 mg/kg/day of carbofuran (equivalent to 0, 0.5, 2.5, 9.0 or 20.0 mg/kg in feed) were administered for eight weeks. During the final four weeks, animals were tested for immunological response to an antigenic challenge and checked for gross abnormalities. Baseline blood counts and body weights were determined during the first 33 days. At day 33, animals were given subcutaneous injections of sheep erythrocytes in Freund's complete adjuvant. Complement-dependent hemolysin titers, hemagglutination titers and immunoglobulin levels (expressed as immunoglobulin/transferrin ratios) were determined 10, 17 and 24 days after immunization, as well as before immunization. No significant changes were observed in either titers or immunoglobulin/transferrin ratios after immunization for any dose, nor were any significant changes observed for total or differential leukocyte counts at any time.

Tuberculin sensitivity was tested by a skin test 10 and 24 days after immunization. Sensitivity (expressed as weal size compared to control group) was significantly decreased for all doses at day 10, but by day 24, only one group (0.49 mg/kg/day) remained significantly decreased.

Animals were sacrificed at day 57, and ratios of organ weight to body weight were calculated for liver, kidney, spleen and adrenal gland. Only the highest dose group (1.05 mg/kg/day) exhibited any significant changes (slight enlargement of the adrenal gland and spleen). No significant body weight changes were observed at any point for any group, and no lesions were found in sections of liver, kidney and adrenal. Lymph node sections were examined for the ability to bind fluoresceinated goat anti-rabbit globulin: the two highest doses showed decreased binding. Significant decreases in germinal centers in stained sections of spleen were found for all dose groups except the 0.49 mg/kg/day group. No significant atrophy was observed in stained thymus sections.

Several changes were observed by the authors to be "large, but not statistically significant." A transient suppression of cell-mediated immunity was observed for carbofuran in the decreased size of the skin weal in a tuberculin test. This by itself is not a strong indication of immunosuppression, and the data presented cannot be interpreted as indicating that carbofuran is significantly immunosuppressive.

Barnett et al. (1980) studied the effect of carbofuran on development and serum immunoglobulin levels in mice. Pregnant female mice (F_2 dihybrid) were

administered daily doses of carbofuran at 0, 0.01 or 0.50 mg/kg bw (43, 23 and 18 animals per group, respectively) in the diet throughout gestation. The doses were 0.02% and 1%, respectively, of the minimum dose causing increased terata or morbidity in pups as determined in a pilot study. Dams in all groups gave birth to approximately equal numbers of offspring. Offspring treatment groups were then monitored for differences in viability and body weight from birth to 800 days (311, 145 and 134 pups for each respective dose group). Serum immunoglobulin levels were measured by radial immunodiffusion for animals sacrificed at 101, 400 and 800 days.

There was no observed effect on viability, average lifespan or incidence of any single cause of death for either carbofuran dose. A transient effect on body weight was observed for pups born to mothers exposed to 0.5 mg/kg/day, (pup mean weight was lower than the control group at birth, 7 and 14 days). No body weight differences were observed at 28 days or for the remainder of the lifespan. No body weight differences were observed for the lower dose animals. However, a follow-up investigation by the same investigator and colleagues (Avery, Spyker-Cranmer and Barnett; unpublished study) appears to reflect a negative response for immunotoxicity.

Measurement of serum IgG_1, IgG_{2a}, IgG_{2b}, IgM and IgA revealed dose-related effects of carbofuran on immunoglobulin levels in one case. Male pups prenatally exposed to the higher dose (0.5 mg/kg/day) exhibited elevation of IgG_1 at 101 and 400 days, but not at 800 days. A transient, non-dose-related effect was seen for females; decreased serum IgG_1 and IgG_{2a} levels were

observed at 101 days, but only in low-dose animals. Additionally, two inconsistent increases in IgA levels in females were observed at 101 days for the high-dose group and at 400 days for the low-dose group. No other significant differences were observed for any other values compared, including IgM and IgG_{2b}. This study has led to the listing of carbofuran as a developmental immunotoxicant (Roberts and Chapman 1981).

Several hematological parameters were evaluated in mice treated with carbofuran on a subacute basis (Gupta et al. 1982). A group of 15 male Swiss mice (four to six weeks old, 20 to 25 g) were given weekly injections of 0.25 mg/kg carbofuran for six weeks. Carbofuran was dissolved in normal saline and injected intraperitoneally. A control group of 15 mice received the same volume of saline. Mice were sacrificed and parameters were measured 18 hours after the sixth injection. Most of the parameters measured were significantly affected by carbofuran treatment, as shown in Table V-4.

The observed changes are consistent with three gross effects, as suggested by the authors. These include liver damage (evidenced by the prolongation of clotting time), bone marrow damage (leading to macrocytic anemia) and possible compensation by the spleen for bone marrow damage.

B. Chronic Toxicity

Chronic administration of carbofuran did not result in any cumulative toxic effects. Rotaru at al. (1981) fed male Wistar rats (initial weight 75 g) carbofuran in the diet for 180 days at levels of 0, 10 or 25 mg/kg (ten animals per group). These levels are approximately 0, 0.49 and

Table V-4 Hematological Parameters of Mice Administered Carbofuran or Normal Saline

Parameter	Normal saline	Carbofuran	% Change
Clotting time	29.5	116[a]	+293
Hemoglobin content in g/100 mL whole blood	13.14	9.1[a]	-31
Total count of RBC/mm^3	10.7×10^6	2.96×10^{6a}	-72
Total count of WBC/mm^3	4,863	7,063[a]	+45
Platelet count/mm^3	$1,348 \times 10^3$	433×10^{3a}	-68
Erythrocyte sedimentation rate (mm/h)	1.6	1.06[a]	-34
Hematocrit value (%)	42.71	17.49[a]	-59
Mean corpuscular volume (μm^3)	39.91	58.3[a]	+46
Mean corpuscular hemoglobin (pg)	12.28	34.88[a]	+184
Mean corpuscular hemoglobin concentration (%)	30.76	47.23[a]	+54
Lymphocytes (% WBC)			
a. Small	55.4	42.4[a]	-23
b. Large	16.8	23.5[a]	+40
Monocytes (% WBC)	5.7	4.9	-14
Basophils (% WBC)	0.9	2.7[a]	+200
Neutrophils (% WBC)	20.3	26.3[a]	+30
Total count of bone marrow cells/femur	8.4×10^6	6.02×10^{6a}	-28

continued-

[a]Value significantly different from control, $P<0.01$; Student's t-test.

Table V-4 - continued

Parameter	Normal saline	Carbofuran	% Change
Erythrocyte precursors of bone marrow/femur	1.2×10^6	0.36×10^{6a}	-70
WBC precursors of bone marrow/femur	6.5×10^6	5.63×10^{6a}	-13
Megakaryocyte precursors of bone marrow/femur	0.7×10^6	ND[b]	--
Weight of spleen (mg)	118.8	113.2	-5
Total count of splenic cells/spleen	2.1×10^8	7.8×10^{8a}	+271

[a]Value significantly different from control, $P<0.01$, Student's t-test.
[b]ND = None detected.

Adapted from Gupta et al. (1982).

1.18 mg/kg/day, calculated using reported final body weights of 287 g and 296 g, respectively, and assuming a daily food consumption of 0.014 kg (Arrington 1972). The animals were examined histopathologically after sacrifice, and the activity of hepatic enzymes was measured. Serum enzymes examined included lactic dehydrogenase (LDH), glutamate dehydrogenase, gamma-glutamyl transpeptidase, glutamate oxalate transaminase, glutamate pyruvate transaminase and alkaline phosphatase.

No differences in weight increase or liver weights were observed between controls and treated animals during the experiment. The minor changes observed in liver morphology seemed to relate more to increased caloric intake associated with feeding ad libitum than to the level of carbofuran administered. The only serum enzyme showing a significant ($P<0.01$) increase was LDH; the increase was observed only at 10 mg/kg carbofuran. Thus, there appeared to be no dose-response relationship for serum enzymes. This study does not clearly define a NOAEL based on hepatic enzyme effects.

Wolfe and Esher (1980) fed old-field mice (Peromyscus polionotus) and cotton mice (P. gossypinus) 100 mg carbofuran/kg for eight months in order to assess toxic effects. This dose level corresponds to approximately 19.6 mg/kg/day for P. polionotus and 12.2 mg/kg/day for P. gossypinus (based on reported body weights of 13.8 g or 29.5 g and reported daily food consumptions of 2.7 or 3.6 g, for each respective strain). Animals were kept as male-female pairs with their pups. Food consumption, mortality, reproduction (number of litters, litter size and percent survival) and age at appearance of selected developmental characters of young were monitored throughout the duration of

the experiment. Male survivors were tested for activity in a behavioral maze at the end of the treatment period. There were no indications of cumulative or delayed effects, except for possible delayed mortality in P. polionotus. The highest NOAEL that can be defined from this study is 19.6 mg/kg/day.

In a one-year dietary study (FMC Corporation 1983), four groups of beagle dogs were administered daily doses of 0, 10, 20 or 500 mg/kg carbofuran in the diet, or approximately 0, 0.25, 0.50 or 12.5 mg/kg/day, assuming a body weight of 16 kg and daily food consumption of 0.4 kg (Arrington 1972). The animals were allowed access to the treated food for two hours each day. Each group consisted of six males and six females. Of the various biochemical, hematological and clinical observations made, the only positive result was depression of plasma cholinesterase at the higher dose levels. No biologically significant effects were observed at the 10-mg/kg (0.25 mg/kg/day) level. At 20 mg/kg (0.50 mg/kg/day), there was a consistent, but not biologically significant depression of plasma cholinesterase in males; therefore, this level is the NOAEL. At 500 mg/kg (12.5 mg/kg/day), there was marked depression of plasma cholinesterase in both males and females and a consistent reduction in erythrocyte cholinesterase.

In a rat study (FMC Corporation 1980d), Charles River CD rats were administered carbofuran at 0, 10, 20 or 100 mg/kg in the diet for two years, corresponding to approximately 0, 0.5, 1.0 or 5 mg/kg/day. Ninety animals per sex per dose were utilized in the study. Parameters observed included body weight, food consumption, behavior, ophthalmoscopy, hematology, blood

chemistry (blood urea nitrogen, serum alkaline phosphatase, serum glutamic pyruvic transaminase, glucose, albumin and cholinesterase determinations), urinalysis and histopathology. The only statistically significant change observed was slightly decreased group mean body weights for males at 100 mg/kg throughout the study. The 10- and 20-mg/kg groups and all females were comparable to controls throughout the study. There were no effects on cholinesterase at 10 or 20 mg/kg, while at 100 mg/kg there was some statistically and biologically significant plasma, red blood cell and brain cholinesterase depression. The NOAEL for this study was determined to be 1.0 mg/kg/day.

In a similar mouse study (FMC Corporation 1980c), Charles River CD-1 mice were fed carbofuran at 0, 20, 125 or 500 mg/kg in the diet, or approximately 0, 3, 18.8 or 75 mg/kg/day. One hundred animals per sex per dose were utilized in this two-year study. Again, body weight, food consumption, behavior, hematology, biochemistry, urinalysis and histopathology were observed. At the highest dose (75 mg/kg/day), there was a significant decrease in body weight up to 78 weeks in females and up to 65 weeks in males. By the end of the experiment, however, there were no statistically significant differences in body weights between treated and control animals, although intermittent reductions in food consumption occurred during the experimental period. At 18.8 and 75 mg/kg/day, statistically significant reductions in brain cholinesterase were observed in both males and females; there were no effects at lower doses. No significant effects on plasma or red blood cell cholinesterase were observed at any of the dose levels during the experiment. This study defines a NOAEL of 3 mg/kg/day.

C. Reproductive/Teratogenic Effects

In a one-year dietary study (FMC Corporation 1983), four groups of beagle dogs were administered daily doses of 0, 10, 20 or 500 mg/kg carbofuran in the diet, or approximately 0, 0.25, 0.50 or 12.5 mg/kg/day, assuming a body weight of 16 kg and daily food consumption of 0.4 kg (Arrington 1972). Each group, initially consisting of six males and six females, was allowed access to the treated food for two hours each day. There was a treatment-related testicular degeneration with some aspermia in four of the five males at the 500 mg/kg (12.5 mg/kg/day) dose and in one of the six males at the 20-mg/kg (0.50 mg/kg/day) dose. In females at the high-dose level, uterine hyperplasia and hydrometria were observed. A NOAEL of 0.50 mg/kg/day was identified for dogs based on the results of this study.

In another study (FMC Corporation 1980b), rats were treated with dietary carbofuran for three generations to ascertain reproductive effects. Weanling Charles River rats were fed 0, 20 or 100 mg/kg dietary carbofuran, corresponding to approximately 0, 1.0 or 5.0 mg/kg/day, based on an assumed body weight of 350 g and daily food consumption of 14 g (Arrington 1972). Generations one and two consisted of 10 males and 20 females per group, and generation three contained 10 males and 24 females. Behavior and survival were observed daily; body weight and food consumption were evaluated weekly. Reproductive parameters evaluated included male and female fertility, gestation time, litter size and growth rates and viability and survival of pups through weaning. All parents and pups of the second mating for each of the three generations were evaluated

histologically. No effect on either female or male fertility or on the length of gestation was observed at either dose level. Litter size and growth were comparable to controls in both treatment groups in all three generations. The viability and survival of pups were largely unaffected by carbofuran, except that survival of the first litter in all three generations in the 100-mg/kg group (5.0 mg/kg/day) was slightly lower by day four of lactation. Therefore, the authors concluded that the NOAEL for carbofuran, using reproductive effects as an endpoint, was 20 mg/kg, corresponding to 1.0 mg/kg/day carbofuran.

Studies on the immunocompetence of mice exposed to carbofuran in utero produced data on the reproductive effects of the compound (Barnett et al. 1980). These results are presented in Table V-5. No significant ($P>0.05$) effects on the number of pups born or on the mortality of pups at 28 or 800 days were observed when the dams were administered either 0.01 or 0.50 mg/kg/day throughout gestation.

The FMC Corporation conducted a complete series of teratology studies with carbofuran in the rat (dietary exposure) and in the rabbit (oral gavage). In a preliminary experiment (FMC Corporation 1980a), pregnant female Charles River CD rats were fed diets containing 0, 20, 60, 120, 160 or 200 mg/kg of carbofuran during gestation days six through nineteen. These dose levels correspond to approximately 0, 1.0, 2.9, 5.8, 7.7 or 9.7 mg/kg/day, assuming a body weight of 0.290 kg and a food consumption of 0.014 kg/day (Arrington 1972). There was 100% survival in all groups. Maternal body weight gain was decreased during the first two days of treatment in the 120, 160 and 200 mg/kg

Table V-5 Mortality of Offspring From Mice Receiving Carbofuran During Gestation

Dose (mg/kg/day)	Number of dams	Number of pups born	% Mortality to 28 days	% Mortality to 800 days
0	43	311	6	33
0.01	23	145	5	29
0.50	18	134	6	27

Adapted from Barnett et al. (1980).

groups. No other clinical symptoms or uterine abnormalities were observed. On the basis of this study, a complete teratology study was conducted (FMC Corporation 1981a). Forty pregnant female Charles River CD rats were fed carbofuran at 0, 20, 60 or 160 mg/kg in the diet (daily doses of 0, 1.0, 2.9 or 7.7 mg/kg bw) during gestation days six through nineteen. One-half of the fetuses were examined for visceral and skeletal abnormalities on gestation day 20. Remaining dams delivered normally and the pups were mothered until lactation day 21. There were no clinical signs of carbofuran toxicity in any group. Food consumption was normal in the 20- and 60-mg/kg groups and was reduced 6% in the 160-mg/kg group. Maternal body weight gains were significantly reduced in the 60- and 160-mg/kg groups during the treatment period; no such effect was seen in the 20-mg/kg group. During the lactation period, mean body weight gain in all treatment groups was normal. There were no effects on length of gestation, parturition, physical appearance of pups or any pup survival indices. The mean pup body weights in the 60 and 160 mg/kg (2.9 and 7.7 mg/kg/day) groups were slightly reduced, but this was attributed to the reduction in maternal body weights in those groups. No terata were observed in any group. On the basis of this study, 20 mg/kg (1.0 mg/kg/day) was determined to be the NOAEL in rats.

In a rabbit study (FMC Corporation 1981b), seven-month-old female New Zealand rabbits were treated (by oral gavage) at 0, 0.12, 0.5 or 2.0 mg/kg/day on days six through eighteen of gestation. One of the dams in the high-dose group died on the eleventh day of gestation from unknown causes. The other animals in that group experienced a 20% reduction in body weight gain during the treatment period. No terata were observed in any of the treatment groups,

and there were no differences in the number of fetuses or litters with developmental or genetic abnormalities in any of the carbofuran-treated groups when compared to the control group. Three dams aborted toward the end of the gestation period, one from each of the treatment groups, but these events were not attributed to carbofuran treatment. Thus, this study indicated no teratogenic effects of carbofuran in rabbits at the dose levels used.

D. Mutagenicity

There have been many studies of the mutagenicity of carbofuran using short-term tests. These are summarized in Table V-6.

Waters et al. (1982) employed seven genotoxicity assays in testing technical-grade carbofuran for mutagenicity. These assays included both prokaryotic and eukaryotic systems and were capable of detecting point mutations and primary DNA damage. Carbofuran produced negative results in five of the seven assays and inconclusive results in the remaining two.

Two bacterial reversion assays were used. Five strains of Salmonella typhimurium, TA98, TA100, TA1535, TA1537 and TA1538, were used in a plate incorporation assay. Escherichia coli strain WP2 was used in a similar reversion assay. At least six doses, up to 10 mg/plate, were tested for each strain, with and without S9 activating system (Aroclor-1254-induced rat). No dose-dependent increase in reversion rate was observed in either assay.

Table V-6 Short-Term Assays for Mutagenicity of Carbofuran

Test	Dose[a]	Results	Reference
Ames (strains)			
TA98,100,1535,1537,1538	up to 10 mg/plate (T)	-,-[b]	Waters et al. 1982
TA98,100,1535,1537,1538	10 µg/plate (?) (T)	-,-[b]	Gentile et al. 1982
TA98,100,1535,1537,1538	10 µg/plate (C)	-,-[b,c]	Gentile et al. 1982
TA98,1538	up to 10 mg/plate	+,+[b]	Moriya et al. 1983
TA100,1535,1537	up to 5 mg/plate	-	Moriya et al. 1983
TA98	NT[d]	+	Shirasu et al. 1982
TA98,100,1537,1538	NT	-,-[b]	Poole et al. 1977
TA98,100	up to 25 µg/plate (A)	-,-[b]	Nelson et al. 1981
Other Tests			
E. coli WP2 reversion	up to 10 mg/plate (T)	-,-[b]	Waters et al. 1982
E. coli WP2 reversion	up to 5 mg/plate (T)	-	Moriya et al. 1983
E. coli WP2 reversion	NT	-,-[b]	Poole et al. 1977
E. coli reversion	NT	-	Simmons et al. 1979
E. coli, B. subtilis differential toxicity	NT	-	Simmons et al. 1979
Yeast mitotic recombination	NT (T)	-	Waters et al. 1982
Yeast gene conversion	over 2-3 logs (T,C)	-,-[b,c]	Gentile et al. 1982

continued-

[a]Grade of carbofuran: (T) = technical, (C) = commercial, (A) = 99.5% analytical.
[b]With metabolic activation by rat S9.
[c]With metabolic activation by plant homogenate.
[d]NT = No test (mixed or ambiguous results).

Table V-6 - continued

Test	Dose[a]	Results	Reference
Yeast mitotic recombination	NT	-	Poole et al. 1977
Z. mays back mutation	2.24 kg/ha (C)	-	Gentile et al. 1982
D. melanogaster recessive lethal	NT (T)	-	Waters et al. 1982
CHO V79 cells	NT	+,-[b]	Wojciechowski et al. 1982
UDS Human lung fibroblasts	5 doses (T)	-,-[b]	Waters et al. 1982
UDS Human fibroblast	NT	-	Simmons et al. 1979

[a]Grade of carbofuran: (T) = technical, (C) = commercial, (A) = 99.5% analytical.
[b]With metabolic activation by rat S9.
[c]With metabolic activation by plant homogenate.
[d]NT = No test (mixed or ambiguous results).

Three eukaryotic assays were used. In sex-linked recessive lethal assay employing Drosophila melanogaster carbofuran elicited a less than 0.2% increase in the spontaneous mutation rate. A mitotic recombination assay in the yeast Saccharomyces cerevisiae (strain D7) revealed no activity for carbofuran at any of five doses, with or without S9 activation. In a variation of Simmon's unscheduled DNA synthesis assay employing WI-38 human lung fibroblast cells, none of the five carbofuran concentrations tested significantly increased ^{3}H-labeled thymidine incorporation into synchronized WI-38 cells, with or without S9.

Two bacterial assays for primary DNA damage also were performed. Both assays depended on the differential toxicity between two bacterial strains, one of which is deficient in the ability to repair DNA. One assay used E. coli strains p3478 and W3110, while the other used Bacillus subtilis strains M45 and H17. Results of both of these assays were inconclusive.

Moriya et al. (1983) tested carbofuran (of unspecified purity) in two bacterial reversion mutagenesis assay systems according to the Ames method. A positive dose-response relationship for S. typhimurium strains TA1538 and TA98 was reported, with no effect caused by the addition of S9 mix. Doses tested for TA1538 were implied to be the same as for TA98. The reversion rate for TA98 was calculated to be 0.0023 revertants per nmole. No increase in reversion rate was observed in carbofuran tests of S. typhimurium strains TA100, TA1535 and TA1537 and of E. coli strain WP2 hcr up to a dose of 5 mg/plate. Testing on the above four strains was implied to have been done with and without S9 mix.

Gentile et al. (1982) conducted mutagenicity tests for ten insecticides using S. typhimurium and S. cerevisiae following plant or animal activation. The plant activating system was the soluble fraction (10 minutes, 1,000 x g supernatant) of homogenized Zea mays plants. Carbofuran was negative for genotoxic properties in all tests.

Wojciechowsk et al. (1982) tested the ability of carbofuran to induce an increase in the frequency of ouabain resistance in CHO V-79 cells. The results indicated that carbofuran (at an unspecified dose level) induced a weak mutagenic response without, but not with, rat liver S-9 activation.

Carbofuran was tested to ascertain its effect on chlorophyll a synthesis and nitrogen fixation in five cyanobacterial forms belonging to the genera Hapalosiphon, Westiellopsis and Calothrix (Kaushik and Venkataraman 1983). No effects were observed in cultures at final concentrations of 1 and 10 mg/L, while minimal inhibition of chlorophyll a was observed in most species at 100 mg/L.

N-Nitrosocarbofuran has also been extensively tested for its mutagenic potential in several short-term assays. These results are summarized in Table V-7.

Nelson et al. (1981) obtained 99.5% pure carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran and prepared N-nitroso derivatives of each. Mutagenicity for all six chemicals was tested using S. typhimurium strains TA98 and TA100, with rat liver S9 fraction activation. The nonnitrosated carbofuran,

Table V-7 Short-Term Assays for Mutagenicity of N-Nitrosocarbofuran

Test	Dose	Results	Reference
Ames (strains)			
TA1535	1.0 to 1,000 µg/plate	+	Lijinsky & Andrews 1979
TA100,1535	0.1 to 100 µg/plate	+,-[a]	Rickard et al. 1982
TA98	0.1 to 100 µg/plate	-,-[a]	Rickard et al. 1982
TA98	2 to 120 ng/plate	-	Nelson et al. 1980
TA100	2 to 120 ng/plate	+	Nelson et al. 1980
TA100	up to 25 µg/plate	+	Nelson et al. 1981
Other Tests			
SCE - CHO cells	10^{-9} to 5 x 10^{-5}M	+	Nelson et al. 1981
CHO cells chromosome aberrations	5 x 10^{-8} to 10^{-5}M	+	Nelson et al. 1981

[a]With rat S9 metabolic activation.

3-hydroxycarbofuran and 3-ketocarbofuran all failed to show mutagenic activity in either the TA98 or TA100 strain, with or without S9 activation, in parallel tests. However, all three of the nitroso derivatives were highly mutagenic without S9 activation in the TA100 but not in the TA98 strain. Addition of S9 fraction decreased mutagenic activity. Nelson et al. (1981) rank the nitroso derivatives of carbofuran and its metabolites among the most mutagenic compounds tested in the TA100 system to date.

E. Carcinogenicity

The FMC Corporation tested carbofuran for carcinogenicity in both rats and mice. In neither case was there a significant increase in tumors of any kind attributed to carbofuran when compared with controls.

In the rat study (FMC Corporation 1980d), Charles River CD rats were administered carbofuran at 0, 10, 20 or 100 mg/kg in the diet for two years, corresponding to approximately 0, 0.5, 1.0 or 5 mg/kg/day. The study utilized 90 animals per sex per dose. Parameters observed included body weight, food consumption, behavior, ophthalmoscopy, hematology, blood chemistry (blood urea nitrogen, serum alkaline phosphatase, serum glutamic pyruvic transaminase, glucose, albumin and cholinesterase determinations), urinalysis and histopathology. The only statistically significant change observed was slightly decreased group mean body weights for males at 100 mg/kg throughout the study. The 10- and 20-mg/kg male groups and all females were comparable to controls throughout the study. There were no effects on cholinesterase at 10 or 20 mg/kg, while at 100 mg/kg some statistically and biologically

significant plasma, red blood cell and brain cholinesterase depression occurred. There were no significant differences between the number of animals without neoplasms, the number with benign neoplasms and the number with malignant neoplasms. In this study, neoplasms and nonneoplastic histologic changes observed were judged to be spontaneous lesions unrelated to the administration of or exposure to carbofuran during the two-year treatment period.

In the mouse study (FMC Corporation 1980c), Charles River CD-1 mice (100 animals per sex per dose) were fed carbofuran at 0, 20, 125 or 500 mg/kg in the diet, or approximately 0, 3, 18.8 or 75 mg/kg/day, for two years. Again, body weight, food consumption, behavior, hematology, biochemistry, urinalysis and histopathology were observed. At the highest dose (75 mg/kg/day), there was a significant decrease in body weight up to 78 weeks in females and up to 65 weeks in males. By the end of the experiment, however, there were no statistically significant differences in body weights between treated and control animals, although some intermittent reductions in food consumption occurred during the experimental period. At 18.8 and 75 mg/kg/day, statistically significant reductions in brain cholinesterase were observed in both males and females; there were no effects at lower doses. No significant effects on plasma or red blood cell cholinesterase were observed at any of dose levels during the experiment. Analysis of tumor incidence in the animals indicated no treatment-related tumorigenic influence or microscopic changes associated with the administration of carbofuran.

Lijinsky and Schmahl (1978) administered N-nitroso derivatives of various agricultural chemicals, including nitrosocarbofuran, to six- to eight-week-old Sprague-Dawley rats. Sixteen male and 16 female rats were administered a solution of nitrosocarbofuran in corn oil at the rate of 66 mg/kg bw by gavage once a week. Treatments were carried out for ten consecutive weeks as a test for carcinogenicity. Three rats died due to liver toxicity after two treatments. The experiment was restarted with a new group of rats, at one-quarter the dose (16.5 mg/kg), once a week for a total of 23 weeks, at which time six rats had died and treatments were terminated. Another group of rats was given a lower dose, 10 mg/kg, by gastric intubation, weekly for ten weeks. A few rats were sacrificed when moribund. All other rats were allowed to survive until spontaneous death. The results of histological examinations for tumors performed on each rat are summarized in Table V-8. Stomach tumors were observed in 17 of the 26 rats given the 16.5-mg/kg dose, and in 3 of the 12 rats given the 10 mg/kg dose. In contrast, none of the 40 control rats given weekly treatments with the oil vehicle alone developed any tumors.

F. Summary

The mode of action of carbofuran in animals is inhibition of acetylcholinesterase, and that enzyme is the most sensitive indicator of the acute effects of the compound. Symptoms of poisoning in animals reflect the inhibition of nerve synaptic acetylcholinesterase. Mammalian LD_{50} values for carbofuran range from 2.0 to 14.1 mg/kg. The toxicity to some birds is somewhat greater.

Table V-8 Survival and Carcinomas in Rats Administered Nitrosocarbofuran by Gavage

No. of weeks	Weekly dose (mg/kg)	Total dose (mg/kg)	Sex[a]	Number of rats surviving at week						Number of rats with stomach carcinomas	All tumors
				0	20	40	60	80	100		
Corn oil control		--	F	40	40	40	35	14	7	0	0
23	16.5	380	M	13	12	12	11	4	0	11	11
			F	13	8	8	6	2	0	6	7
10	10	100	F	12	8	4	3	3	1	3	3

[a]M = male; F = female.

Adapted from Lijinsky and Schmahl (1978).

Other acute effects on the immune system and the blood have been reported, but these effects are not severe and are reversible when exposure to carbofuran is terminated.

No significant increases in tumors were observed in carbofuran tests for carcinogenicity in both rats and mice.

Many short-term assays of the mutagenic potential of carbofuran have been conducted. Most tests were negative, although there were a few positive results, particularly in S. typhimurium strains TA98, TA1538 and CHO V-79 cells. The N-nitroso derivative of carbofuran, which results from the chemical nitrosation of carbofuran by nitrite under acidic conditions, is both a potent mutagen and causes stomach cancer in rats. However, there is no evidence for the environmental or biological formation of this compound. Bioassays to determine the possible carcinogenicity of carbofuran in rats and mice have been negative.

Carbofuran does not appear to cause any teratological effects in laboratory animals, although it has caused some effects on the reproductive system. Aspermia and testicular degeneration in dogs and some small decreases in pup survival in rats have been observed, but only at doses higher than the NOAEL based on cholinesterase inhibition.

VI. HEALTH EFFECTS IN HUMANS

A. Clinical Case Studies

The only reports in the literature of human exposure to carbofuran under controlled conditions were described by FMC Corporation in its pesticide registration petition (FMC Corporation 1977). Human subjects were exposed to oral and dermal carbofuran doses in an experimental setting. A progression of oral doses beginning with 0.05 mg/kg was established for administration to healthy males (two subjects per dose). The compound was administered as a single dose following breakfast. At the lowest dose, 0.05 mg/kg, no symptoms were observed. At 0.10 mg/kg, symptoms included headache and a feeling of light-headedness, which the investigators indicated may have been unrelated to the treatment (no further explanation given). At 0.25 mg/kg, symptoms of acetylcholinesterase depression were observed (dry mouth, salivation, diaphoresis, abdominal pain, drowsiness, dizziness, anxiety and vomiting). As a result, no higher doses were used. The lowest dose used (0.05 mg/kg) can be defined as the NOAEL in this study.

In dermal studies, two healthy males were treated after breakfast with carbofuran at doses beginning with 0.5 mg/kg/cm^2 in artificial sweat. The human response to carbofuran challenge appeared to be related to exercise, temperature and relative humidity. At 2 mg/kg under high temperature (85° to 95°F) and humidity (68% to 85%) with mild exercise, symptoms of cholinesterase depression were elicited. These symptoms correlated with 44% or more depression in erythrocyte cholinesterase. However, no symptoms were elicited

by doses up to 32 mg/kg at low temperatures (70° to 75°F) and humidity (35% to 40%). Three days of sequential dosing at 1 mg/kg or less resulted in no cumulative cholinesterase depression.

FMC conducted an additional study comparing cholinesterase inhibition and effects of Furadan 4F[a] and FMC 35001 4EC (Arnold 1978). Healthy adult human males, median age 36, received a dermal application of carbofuran during a four-hour exposure period (after which the compound was washed off). The observed symptoms were those of cholinesterase inhibition (nausea, stomach cramps, vomiting, dizziness, weakness, dry lips, swollen tongue, increased salivation and tremors). Doses with the carbofuran formulation up to 4 mg/kg for four hours resulted in a dose-related decrease in erythrocyte cholinesterase (15% at 0.5 mg/kg, 25% at 1.0 mg/kg, 41% at 2.0 mg/kg, 55% at 4.0 mg/kg). No dose-response relationship was observed for plasma cholinesterase depression in the same dose range.

Several cases of adverse effects in applicators and formulators using carbofuran have been reported (Tobin 1970). Symptoms from inhalation exposure included mild and reversible symptoms of acetylcholinesterase depression such as malaise, hyperhydrosis, light-headedness, nausea, blurring of vision, hypersalivation and vomiting. These symptoms were reported observed within two-hours of exposure and the affected persons were reported completely recovered within five to six hours after exposure when no treatment was administered or within one-half hour after treatment with atropine. Symptoms

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[a]Registered trademark.

that may occur with more severe poisoning include chest tightness, muscular twitching, convulsions and coma.

Two cases of direct adverse effect of carbofuran on the eye (burning and blurred vision) without systemic intoxication were also reported by Tobin (1970). The time between onset of symptoms and recovery was not recorded for one case. The other was reported completely recovered within ten minutes after taking two tablets of atropine.

The N-methylcarbamates, of which carbofuran is an example, inhibit several aliesterases. However, there is no known toxic manifestation of this effect (Tobin 1970).

B. Epidemiological Studies

There were no reports in the literature of long-term or epidemiological studies of carbofuran in humans.

C. High-Risk Subpopulations

Because of their direct exposure to carbofuran in pest control operations, formulators and applicators may be at higher risk. As cited previously, several cases of adverse effects in applicators and formulators using carbofuran have been reported (Tobin 1970).

D. Summary

Human exposure to carbofuran results in symptoms of acetylcholinesterase depression. In both controlled dose studies and incidents of environmental exposure, symptoms included nausea, vomiting, light-headedness, dry lips, drowsiness or weakness, increased salivation and tremors. In a controlled study, a NOAEL of 0.05 mg/kg was established when administered as a single dose.

VII. MECHANISM OF TOXICITY

A. Acetylcholinesterase Inhibition

As described earlier, carbofuran is a potent direct inhibitor (i.e., it does not require metabolic alteration to become active) of the enzyme acetylcholinesterase (Tobin 1970).

Carbofuran, like the organophosphorous and other carbamate insecticides, acts on the nervous system by inhibiting acetylcholinesterase at the synapse (O'Brien 1976). Messages are transmitted across the nerve synapse by transmitter substances, such as acetylcholine in cholinergic junctions. When an impulse has passed, acetylcholinesterase acts on the acetylcholine to remove it from the system. The carbamates, including carbofuran, owe their activity to configurational similarity to acetylcholine and interaction with the active sites of acetylcholinesterase. Carbofuran serves as a substrate for the enzyme, carbamylating the catalytic site and thereby inactivating the acetylcholinesterase.

The structure of carbofuran includes a bulky side chain that is capable of interacting with one or more cholinesterase binding sites located at an appropriate distance from the carbamyl group to accommodate the enzyme (O'Brien 1976). The carbamylated acetylcholinesterase is less stable than the phosphorylated enzyme that results from reaction with organophosphate insecticides. Thus, its effect is more quickly reversible, and symptomatic recovery is more rapid.

B. Interactions

No studies in mammals of carbofuran interactions with other chemicals were found in the literature. Dieter and Ludke (1978) reported that morsodren (as methyl mercury) had a slight potentiating effect in quail. Methylene dioxyphenyl compounds interact synergistically with other carbamates, but no data on carbofuran were located.

C. Summary

The mechanism of action of carbofuran is that of its class of insecticides, the carbamates. Inhibition of acetylcholinesterase by carbofuran has been amply demonstrated, and it appears to behave as a conventional member of its group. There are no data on interactions of carbofuran with other compounds, although methylene dioxyphenyl compounds are potent synergists with other carbamates.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals which do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

Quantification of Noncarcinogenic Effects

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), (formerly called the Acceptable Daily Intake (ADI)), is calculated. The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious health effects, even if exposure occurs over a lifetime. The RfD is derived from a No-Observed-Adverse-Effect-Level (NOAEL), or Lowest-Observed-Adverse-Effect-Level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s). The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{Uncertainty Factor(s)}} = ___ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based on professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the

Office of Drinking Water (ODW) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS 1977, 1980) as follows:

- An uncertainty factor of 10 is generally used when good chronic, or subchronic human exposure data identifying a NOAEL are available and are supported by good chronic, or subchronic toxicity data in other species.

- An uncertainty factor of 100 is generally used when good chronic toxicity data identifying a NOAEL are available for one or more animal species (and human data are not available), or when good chronic, or subchronic toxicity data identifying a LOAEL in humans are available.

- An uncertainty factor of 1,000 is generally used when limited or incomplete chronic, or subchronic toxicity data are available, or when good chronic, or subchronic toxicity data identify a LOAEL, but not a NOAEL for one or more animal species are available.

The uncertainty factor used for a specific risk assessment is based principally on scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving a RfD, the significance of the adverse health effect and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{(\text{RfD}) \times (\text{Body Weight in kg})}{\text{Drinking Water Volume in L/day}} = ___ \text{ mg/L}$$

where:

Body weight = assumed to be 70 kg for adult

Drinking water volume = assumed to be 2 liters per day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (One-day, Ten-day and Longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using a similar equation to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{UF}) \times (___ \text{ L/day})} = ___ \text{ mg/L}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. One-day HA for a 10-kg child ingesting 1 L water per day.
2. Ten-day HA for a 10-kg child ingesting 1 L water per day.
3. Longer-term HA for a 10-kg child ingesting 1 L water per day.
4. Longer-term HA for a 70-kg adult ingesting 2 L water per day.

The One-day HA calculated for a 10-kg child assumes a single acute exposure to the chemical and is generally derived from a study of less than seven days duration. The Ten-day HA assumes a limited exposure period of one to two weeks and is generally derived from a study of less than 30-days duration. A Longer-term HA is derived for both the 10-kg child and a 70-kg adult and assumes an exposure period of approximately seven years (or 10% of an individual's lifetime). A Longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

Quantification of Carcinogenic Effects

The EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

- Group A: Known Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

- Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

- Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.

- Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.

- Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimate excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually comes from lifetime exposure studies in animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the

average adult human body weight is 70 kg and that the average water consumption of an adult human is two liters of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure via ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any others. Because each model is based upon differing assumptions, the estimates which were derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in

experimental animals or humans. Dose response data usually are available only for high levels of exposure, not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Noncarcinogenic Effects

Carbofuran, a typical carbamate insecticide, is absorbed rapidly after oral or dermal administration. Rats absorbed 63% of an oral dose from the stomach in 30 minutes and 72% of a dermal dose in 15 minutes (Ahdaya et al. 1981, Shah et al. 1981). Excretion was similarly rapid, with 75% of an oral dose being excreted within 24 hours after administration (Dorough 1968). Urinary excretion was the principal path of elimination.

The oral LD_{50} for carbofuran in mammals ranged from 8.0 to 14.1 mg/kg in rodents (Fahmy et al. 1970, Midwest Research Institute 1976). Chickens were more tolerant, with LD_{50} values ranging from 25 to 39 mg/kg. Other avian species were more sensitive; LD_{50} values ranged from 0.2 to 8.0 mg/kg (Midwest Research Institute 1976, Tucker and Crabtree 1970, FMC Corporation 1972).

1. Short-Term Exposure

Cambon et al. (1979) administered carbofuran to groups of pregnant Sprague-Dawley rats on gestation day 18. Eight animals per group were

administered either 0.05, 0.25 or 2.5 mg/kg carbofuran by gastric intubation. The rats were killed 1, 5 or 24 hours after dosing and the activity of acetylcholinesterase was measured in both fetal and maternal blood, brain and liver. Toxic signs appeared at the highest dose (2.5 mg/kg) within five minutes, and mortality was high in this group. The activity of acetylcholinesterase was significantly ($P<0.05$) reduced in all maternal and fetal tissue samples taken from surviving dams one hour after treatment with 2.5 mg/kg carbofuran. For lower doses, inhibition was found only in some tissues one hour after treatment. There was a semiquantitative correlation between acetylcholinesterase inhibition in blood and in brain (nervous system). Differential inhibition of isoenzymes of acetylcholinesterase was observed in both maternal and fetal brain (Cambon et al. 1980). This study defines a LOAEL at 0.05 mg/kg for a single dose, based on inhibition of maternal and fetal blood acetylcholinesterase and maternal liver acetylcholinesterase.

FMC Corporation (1977) conducted a controlled carbofuran exposure experiment using humans. A progression of oral doses beginning with 0.05 mg/kg was established for administration to healthy males (two subjects per dose). The compound was administered as a single dose following breakfast. At the lowest dose, 0.05 mg/kg, no symptoms were observed. At 0.10 mg/kg, symptoms included headache and a feeling of light-headedness, perhaps unrelated to the treatment. At 0.25 mg/kg, symptoms of acetylcholinesterase depression were observed (dry mouth, salivation, diaphoresis, abdominal pain, drowsiness, dizziness, anxiety and vomiting). As a result, no higher doses were used. The lowest dose used (0.05 mg/kg) can be defined as the NOAEL in this study.

2. Long-Term Exposure

Rotaru et al. (1981) fed male Wistar rats (initial weight 75 g) carbofuran in the diet for 180 days at levels of 0, 10 or 25 mg/kg (ten animals per group). These levels are approximately 0, 0.49 or 1.18 mg/kg/day, calculated using reported final body weights of 287 g and 296 g, respectively, and assuming daily food consumption of 0.014 kg (Arrington 1972). The animals were examined histopathologically after sacrifice and the activity of hepatic enzymes was measured. Serum enzymes examined included lactic dehydrogenase (LDH), glutamate dehydrogenase, gamma-glutamyl transpeptidase, glutamate oxalate transaminase, glutamate pyruvate transaminase, and alkaline phosphatase.

No differences in weight increase or liver weights between controls and treated animals were observed during the experiment. The only serum enzyme showing a significant ($P<0.01$) increase was LDH, and this increase was observed only at 10 mg/kg (0.49 mg/kg/day) carbofuran. Thus, there was no apparent dose-response relationship for this effect. This study does not clearly define a NOAEL based on hepatic enzyme effects. There was a significant increase in LDH at 0.49 mg/kg/day, but not at 1.18 mg/kg/day.

Wolfe and Esher (1980) fed old-field mice (Peromyscus polionotus) and cotton mice (P. gossypinus) 100 mg carbofuran/kg food for eight months in order to assess toxic effects. This dose level corresponds to approximately 19.6 mg/kg/day for P. polionotus and 12.2 mg/kg/day for P. gossypinus (based

on reported body weights of 13.8 g or 29.5 g and reported daily food consumptions of 2.7 or 3.6 g, for each respective strain). Food consumption, mortality, reproduction and age at appearance of selected developmental characters of young were monitored throughout the duration of the experiment. Male survivors were tested for activity in a behavioral maze at the end of the treatment period. There were no indications of cumulative or possible delayed effects, except for possible increased mortality in P. polionotus. The highest NOAEL that can be defined from this study is 19.6 mg/kg/day.

In a study reported by FMC Corporation (1980d), Charles River CD rats were administered carbofuran at 0, 10, 20 or 100 mg/kg in the diet for two years, equivalent to approximately 0, 0.5, 1.0 or 5 mg/kg/day. Ninety animals per sex per dose were utilized in the study. Parameters observed included body weight, food consumption, behavior, ophthalmoscopy, hematology, blood chemistry (blood urea nitrogen, serum alkaline phosphatase, serum glutamic pyruvic transaminase, glucose, albumin and cholinesterase), urinalysis and histopathology. The only statistically significant change observed was slightly decreased mean body weights for males at 100 mg/kg throughout the study. The 10- and 20-mg/kg groups and all females were comparable to controls throughout the study. There were no effects on cholinesterase at 10 or 20 mg/kg, while at 100 mg/kg, some statistically and biologically significant inhibition of plasma, red blood cell and brain cholinesterase occurred. Neoplasms and nonneoplastic histologic changes observed in the animals were judged to be spontaneous lesions unrelated to carbofuran exposure during the two-year treatment period. The 20-mg/kg carbofuran diet (1.0 mg/kg/day) is the NOAEL for this study.

In a similar study in mice (FMC Corporation 1980c), Charles River CD-1 mice were fed carbofuran at 0, 20, 125 or 500 mg/kg in the diet, approximately 0, 3, 18.8 or 75 mg/kg/day. One hundred animals per sex per dose were utilized in this two-year study. Body weight, food consumption, behavior, hematology, biochemistry, urinalysis and histopathology were observed. At the highest dose (75 mg/kg bw), there was a significant decrease in body weight up to 78 weeks in females and up to 65 weeks in males. By the end of the experiment, however, there were no statistically significant differences in body weights between treated and control animals, although some intermittent reductions in food consumption occurred during the experimental period. At 18.8 and 75 mg/kg bw, statistically significant reductions in brain cholinesterase were observed in both males and females; there were no effects at lower doses. No significant effects on plasma or red blood cell cholinesterase were observed at any dose level during the experiment. Analysis of tumor incidence in the animals indicated no treatment-related tumorigenic influence or microscopic changes in organs in any of the carbofuran-treated mice. This study defines a NOAEL of 20 mg/kg food (3.0 mg/kg/day).

In a one-year feeding study in dogs (FMC Corporation 1983), four groups of beagles were administered daily doses of 0, 10, 20 and 500 mg/kg carbofuran in the diet, equivalent to approximately 0, 0.25, 0.50 or 12.5 mg/kg/day, assuming a body weight of 16 kg and daily food consumption of 0.4 kg (Arrington 1972). Each group of animals, consisting of six males and six females, was allowed access to the treated food for two hours each day.

Of the various biochemical, hematological and clinical observations made, the only positive result was depression of plasma cholinesterase at the two highest dose levels. No biologically significant effects were observed at the 10-mg/kg (0.25 mg/kg/day) level. At 20 mg/kg (0.50 mg/kg/day), there was a consistent, statistically significant depression of plasma cholinesterase in males, but this was not judged to be biologically significant. At 500 mg/kg (12.5 mg/kg/day), marked depression of plasma cholinesterase occurred in both males and females along with a consistent reduction in erythrocyte cholinesterase. Moreover, four of the five males at that dose and one of the six males at the 20-mg/kg (0.50 mg/kg/day) dose experienced a treatment-related testicular degeneration with some aspermia. In females at the high-dose level, uterine hyperplasia and hydrometria were observed. This study defines a NOAEL of 20 mg/kg (0.50 mg/kg/day) for carbofuran in dogs.

B. Quantification of Noncarcinogenic Effects

1. One-Day Health Advisory

The study by FMC Corporation (1977) has been selected to serve as the basis for calculating of the One-day HA for children. This study was chosen because it provides an oral, single-dose exposure to several levels of carbofuran in humans. The NOAEL observed in this study is 0.05 mg/kg, based on the absence of symptoms of acetylcholinesterase inhibition.

Assumptions regarding absorption are not required in the One-day HA calculations because the NOAEL is expressed in terms of an intake dose rather than an absorbed dose.

The One-day HA for the 10-kg child is calculated as follows:

$$\text{One-day HA} = \frac{(0.05 \text{ mg/kg/day})\ (10 \text{ kg})}{(1 \text{ L/day})\ (10)} = 0.05 \text{ mg/L}$$

where:

0.05 mg/kg/day = NOAEL, based on absence of symptoms of acetylcholinesterase inhibition in humans exposed to a single oral dose of carbofuran (FMC Corporation 1977).

10 kg = assumed weight of a child.

1 L/day = assumed water consumption by a 10-kg child.

10 = uncertainty factor. This uncertainty factor was selected in accordance with NAS/ODW guidelines for use of a NOAEL from a study in humans.

2. Ten-Day Health Advisory

There were no studies involving short-term exposure (one to two weeks) suitable for calculation of a Ten-day HA value found in the literature. Because of the rapidly reversible toxic effects of low doses of carbofuran and the absence of evidence of cumulative toxicity, the One-day HA value for a 10-kg child can also serve as the Ten-day HA value. This value is also identical to the Longer-term HA.

3. Longer-Term Health Advisory

The study by FMC Corporation (1983) has been selected to serve as the basis for the Longer-term HA. This study identified 0.50 mg/kg/day as the NOAEL, based on statistically but not biologically significant plasma cholinesterase depression and testicular degeneration in male beagle dogs. Other chronic studies involving rats (FMC Corporation 1980d) and mice (FMC Corporation 1980c) defined NOAELs at higher levels (1.0 and 3.0 mg/kg/day, respectively). Because of the nature of this chemical (a cholinesterase inhibitor), the acute human study by FMC (1977) was also taken into consideration when computing this HA.

The Longer-term HA for the 10-kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(0.50 \text{ mg/kg/day})\ (10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.05 \text{ mg/L } (50 \text{ µg/L})$$

where:

0.5 mg/kg/day = NOAEL, based on absence of acetylcholinesterase inhibition and testicular degeneration in beagle dogs exposed to carbofuran via the diet for one year.

10 kg = assumed weight of a child.

100 = uncertainty factor; this uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from a study in animals is employed.

1 L/day = assumed water consumption by a 10-kg child.

Similarly, the Longer-term HA for a 70-kg adult consuming 2 L/day of water is calculated as follows:

$$\text{Longer-term HA} = \frac{(0.50 \text{ mg/kg/day}) \ (70 \text{ kg})}{(100) \ (2 \text{ L/day})} = 0.18 \text{ mg/L } (180 \text{ ug/L})$$

4. Reference Dose and Drinking Water Equivalent Level

The one-year dog study by FMC Corporation (1983) as described above, has also been selected to serve as the basis for calculation of the DWEL.

Using this study, the DWEL is derived as follows:

Step 1: Determination of RfD

$$\text{RfD} = \frac{(0.50 \text{ mg/kg/day})}{(100)} = 0.005 \text{ mg/kg/day}$$

where:

0.50 mg/kg/day = NOAEL, based on absence of acetylcholinesterase inhibition and testicular degeneration in beagle dogs exposed to carbofuran via the diet for one year.

100 = uncertainty factor; this uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from a study in animals is employed.

Step 2: Determination of DWEL

$$DWEL = \frac{(0.005 \text{ mg/kg/day}) \ (70 \text{ kg})}{2 \text{ L/day}} = 0.18 \text{ mg/L } (180 \mu g/L)$$

where:

0.005 mg/kg/day = RfD

70 kg = assumed weight of an adult.

2 L/day = assumed water consumption by a 70-kg adult.

The DWEL derived from the dog study is supported further by evidence from the two-year rat feeding study (FMC Corporation 1980d) in which identical RfD (0.005 mg/kg/day) and DWEL (0.18 mg/L) values would be obtained. In this study, groups of rats were fed 0, 10, 20 or 100 mg carbofuran/kg in the diet. A NOAEL of 1 mg/kg/day (the middle treatment dose) was identified, to which an uncertainty factor of 200 was applied to protect against cholinesterase depression and systemic effects. This larger than usual uncertainty factor was used in light of the data generated in the single exposure human volunteer study described above (FMC Corporation 1977). Again, it is assumed that the protected individual is a 70-kg adult consuming two liters of water per day.

This DWEL assumes that the only source of exposure is via drinking water. If there are adequate data to indicate exposure may occur by other routes (food, air, work place), the DWEL may be reduced accordingly so the total daily dose from all sources does not exceed the RfD.

C. Carcinogenic Effects

The FMC Corporation tested carbofuran for carcinogenicity in both rats (FMC Corporation 1980d) and mice (FMC Corporation 1980c). In the two-year rat study, all neoplasms or nonneoplastic histologic changes observed were judged to be unrelated to the exposure to carbofuran. In the two-year mouse study, the analysis of tumor incidence indicated no treatment-related tumorigenic influence in the carbofuran-treated mice.

A number of short-term assays to assess the mutagenic potential of carbofuran have been performed. In most tests, the results have been negative.

The International Agency for Research on Cancer (IARC) has not evaluated the carcinogenic potential of carbofuran (WHO 1982). Applying the criteria described in EPA's proposed guidelines for assessment of carcinogenic risk (USEPA 1986), the Agency has classified carbofuran in Group E: no evidence of carcinogenicity in humans. This category is used for agents that show no evidence of carcinogenicity in at least two adequate animal tests in different species or in both epidemiologic and animal studies.

D. Quantification of Carcinogenic Effects

Carcinogenic effects are not quantified since there are no data indicating that carbofuran is carcinogenic.

E. Existing Guidelines and Standards

Recommended or established standards for carbofuran include occupational exposure limits, tolerances in or on agricultural commodities and acceptable daily intakes. The threshold limit value (TLV) on a time-weighted average basis is established at 0.1 mg/m^3 (ACGIH 1980). The NAS (1983) stated that insufficient information exists to permit calculation of a suggested no-adverse-response level (SNARL) or to assess the possibility of chronic exposure hazards. Most toxicological data on carbofuran, however, are classified as Confidential Business Information and were not available to NAS for their evaluation. The USEPA, Office of Pesticide Programs (OPP) has established tolerances for carbofuran in or on raw agricultural commodities (CFR 1985). These tolerances are based on an Acceptable Daily Intake (ADI) of 0.005 mg/kg/day, also referred to as the Reference Dose (RfD). The World Health Organization (WHO) calculated an ADI of 0.01 mg/kg/day for carbofuran (Vettorazzi and Van den Hurk 1985).

F. Special Considerations

The following special considerations are identified for their potential impact on the risk assessment processes.

1. High-Risk Populations

Because of their direct exposure to carbofuran in pest control operations, formulators and applicators may experience increased exposure and, therefore, be at a higher risk. Several cases of adverse effects in such individuals have been reported (Tobin 1970). These effects, however, were only mild symptoms of acetylcholinesterase depression, which were rapidly reversed when exposure ceased.

2. Interactions

Although studies on synergistic effects of methylene dioxyphenyl compounds on carbofuran have not been reported, these effects might be anticipated by analogy with other carbamate insecticides. No antagonistic effects have been identified.

3. Beneficial Effects

No beneficial effects have been identified.

G. Summary

The recommended values for the One-Day, Ten-day and Longer-term HAs and the DWEL are summarized in Table VIII-1.

TABLE VIII-1 Summary of Quantification of Toxicological Effects for Carbofuran

Value	Drinking water concentration (mg/L)	Reference
One-day HA for 10-kg child	0.05	FMC Corporation 1977
Ten-day HA for 10-kg child	0.05[a]	FMC Corporation 1977
Longer-term HA for 10-kg child	0.05	FMC Corporation 1983
Longer-term HA for 70-kg adult	0.18	FMC Corporation 1983
DWEL (70-kg adult)	0.18	FMC Corporation 1983

[a]One-day HA was taken to be an estimate of the Ten-day HA since inadequate short-term dose-response data were found.

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